REPORT RESUMES

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MEDICAL LABORATORY ASSISTANT, A SUGGESTED GUIDE FOR A TRAINING PROGRAM.

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INFORMATION IS GIVEN TO ASSIST IN ORGANIZING AND ADMINISTERING A TRAINING PROGRAM FOR MEDICAL LABORATORY ASSISTANTS IN A VARIETY OF SETTINGS AND TO PROVIDE GUIDANCE IN ESTABLISHING NEW PROGRAMS AND IN EVALUATING EXISTING ONES. THE MATERIAL WAS PREPARED UNDER THE DIRECTION OF THE NATIONAL COMMITTEE FOR CAREERS IN MEDICAL TECHNOLOGY. PATHOLOGISTS AND MEDICAL TECHNOLOGISTS PARTICIPATED IN THE ORGANIZATIONAL AND DEVELOPMENTAL STAGES. ALL MATERIAL WAS REVIEWED BY A REPRESENTATIVE NATIONAL GROUP OF EXPERT CONSULTANTS IN THE FIELD OF LABORATORY MEDICINE. THE 12-MONTH PROGRAM WAS DESIGNED FOR HIGH SCHOOL GRADUATES OR THEIR EQUIVALENT TO BE ADMINISTERED BY A TEACHING STAFF COMPOSED OF A NATIONAL DIRECTOR, A TEACHING SUPERVISOR, AND INSTRUCTORS. AN OUTLINE OF INFORMATIONAL MATERIAL TO BE PRESENTED IN THE CLASSROOM, LABORATORY PROCEDURES TO BE DEMONSTRATED AND THEN PERFORMED AS DIRECT EXERCISES BY THE STUDENTS, AS WELL AS RELEVANT BIBLIOGRAPHIES, AUDIOVISUAL AIDS, AND STUDY QUESTIONS ARE PRESENTED FOR THE FOLLOWING UNITS -- (1) ORIENTATION TO THE CLINICAL LABORATORY, (2) BACTERIOLOGY, (3) SEROLOGY, (4) PARASITOLOGY, (5) HEMATOLOGY, (6) CLINICAL CHEMISTRY, (7) BLOOD BANKING, (8) ROUTINE ANALYSIS, AND (9) BASAL METABOLISM -- ELECTROCARDIOGRAPHY. THIS DOCUMENT IS AVAILABLE AS GPO NUMBER FS 5.287--87017 FOR 60 CENTS FROM SUPERINTENDENT OF DOCUMENTS, U.S. GOVERNMENT PRINTING OFFICE, WASHINGTON, D.C. 20402. (PS)

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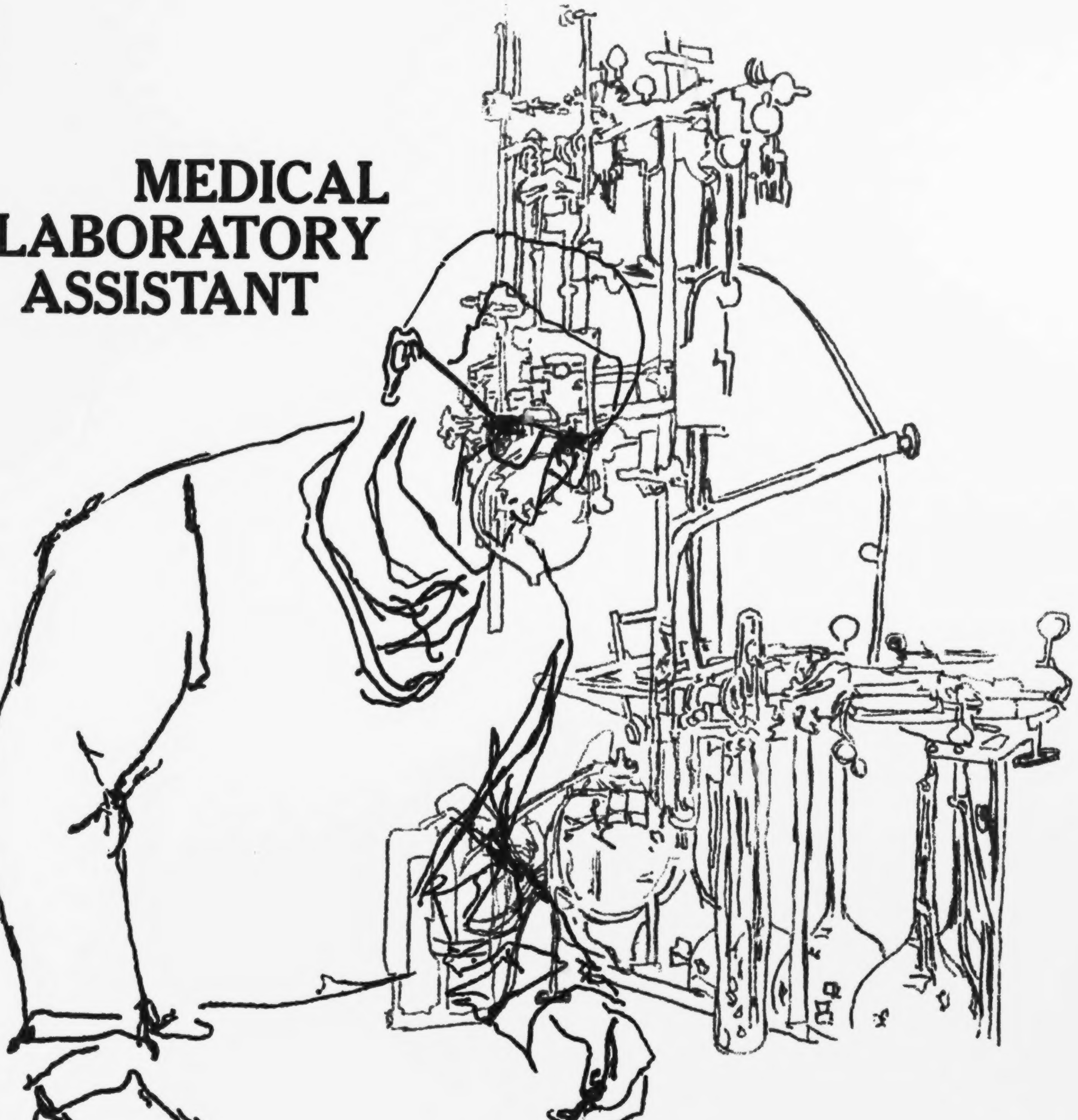

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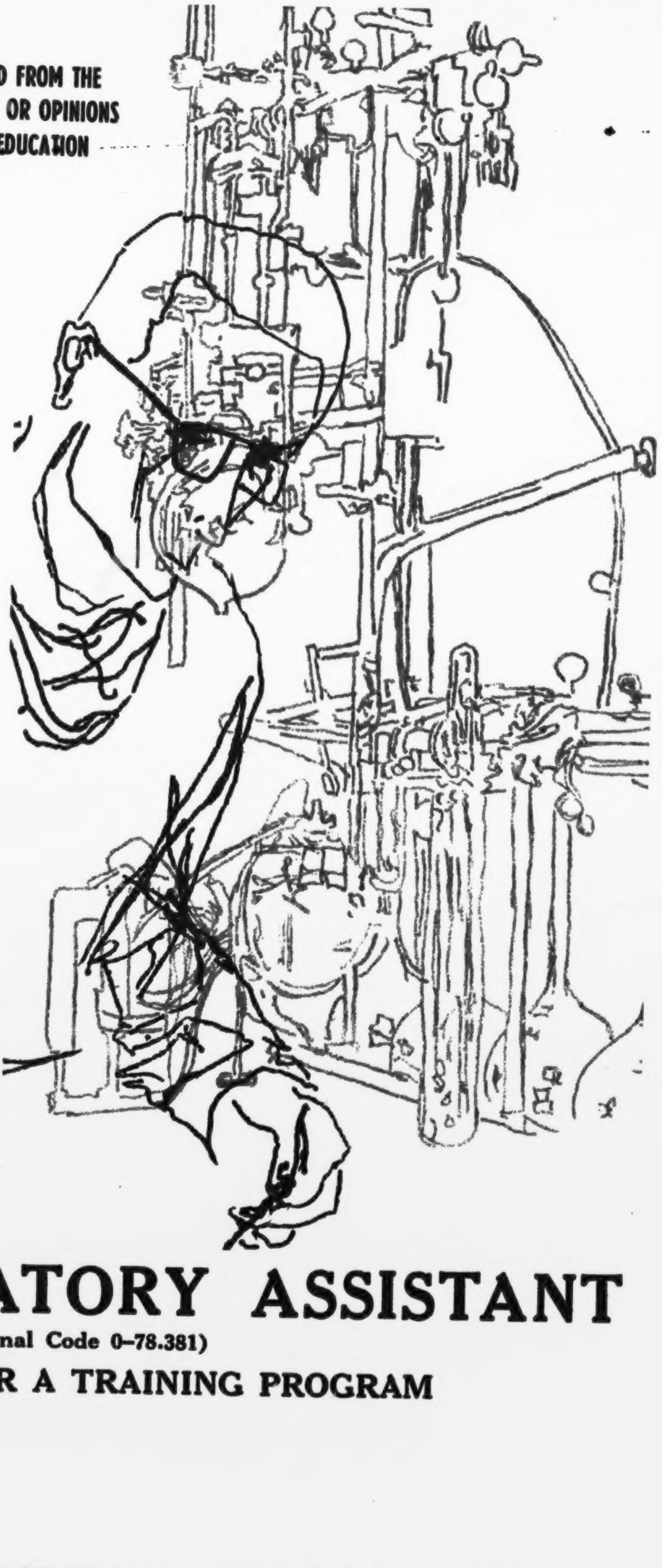

MEDICAL LABORATORY ASSISTANT

(D.O.T. Occupational Code 0–78.381)

A SUGGESTED GUIDE FOR A TRAINING PROGRAM

OFFICE OF EDUCATION/U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
HAROLD HOWE II, *Commissioner* JOHN W. GARDNER, *Secretary*

Manpower Development and Training Program

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FOREWORD

COMPETENTLY TRAINED medical laboratory assistants are urgently needed today in communities across the country to help perform vital life-saving tests and procedures for detecting disease and safeguarding health. Young men and women with a high school education or its equivalent, who have an interest in science and medicine, can learn the necessary skills through the 12-month medically supervised program suggested in this publication and help to meet the great demand for technical manpower in hospital and clinic laboratories.

Laboratory assistants work under the direct supervision of the medical technologist, and a pathologist or other qualified physician, performing routine procedures in bacteriology, blood banking, chemistry, hematology, parasitology, serology, and urinalysis. They are employed primarily in hospital laboratories, but opportunities also are available in public and private clinical laboratories, physicians' offices, public health agencies, and industrial or pharmaceutical medical laboratories. The shortage of individuals with knowledge and training adequate to assist the professional medical technologist in the laboratory makes it probable that training programs suggested in this guide might be established in many communities from coast to coast.

With the rapid growth in training programs set up under medical auspices to meet the need for laboratory assistants, this guide should prove to be useful in providing comparable instructions and skills to young people in all parts of the country and should enable them to perform their tasks capably and efficiently.

This guide provides an outline of informational material to be presented in the classroom, together with laboratory procedures to be demonstrated and then performed as direct exercises by the students, as well as relevant bibliographies, audiovisual aids, and study questions. Also included is information to assist in organizing and administering a training program for medical laboratory assistants in a variety of settings, to provide guidance in establishing new programs, and in evaluating existing ones.

The material was prepared under the direction of the National Committee for Careers in Medical Technology under a contractual arrangement with the Division of Vocational and Technical Education, U.S. Office of Education. Pathologists and medical technologists, most of whom are professionally associated with laboratory assistant training programs, participated in the organizational and developmental stages. All material was reviewed by a representative national group of expert consultants in the field of laboratory medicine.

WALTER M. ARNOLD,
Assistant Commissioner for
Vocational and Technical Education.

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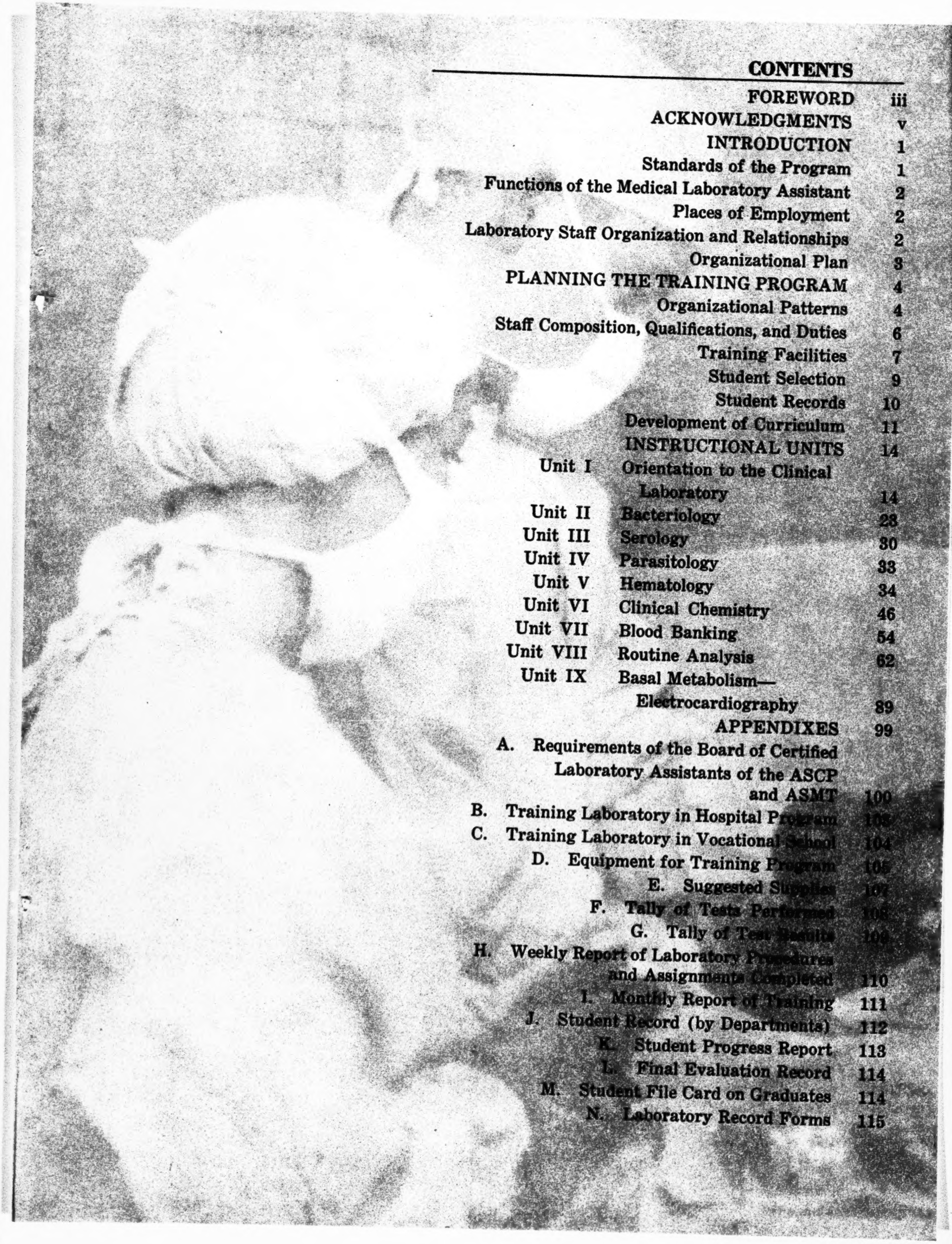

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INTRODUCTION

The tremendous advances in modern medicine have resulted in an ever-increasing demand for vital diagnostic laboratory tests. These tests are often the final determining factor as to the presence and extent, or absence, of cancer, tuberculosis, diabetes, poliomyelitis, and other diseases. The complexities of laboratory science today require a trained staff at various levels of knowledge and skill to carry out both the simple and the intricate analyses which often spell life or death to the patient.

Organizational echelons have developed in the laboratory in recent years, similar to those for nursing services, to facilitate the orderly and effective handling of the multitudinous laboratory procedures. Under the direction of qualified physicians, the laboratory staff includes a department chief, specialists, general duty medical technologists (corresponding to general duty nurses), laboratory assistants (corresponding in some ways to the practical nurse), and sometimes laboratory attendants or aides (operating on the level of nurse's aides).

Each of the echelons in the medical laboratory—from the pathologist-director with 13 years of professional training, and the medical technologist with four years of college science and hospital training, to the laboratory assistant with 1 year of post-high school study—has one objective in common with the others: the patient's welfare. No matter how difficult or how simple the tasks performed by each member of the laboratory team may be, they all affect the well-being of the patient.

The upper echelons of the medical laboratory team—the pathologist and the medical technologist—are trained in approved schools under curriculums standardized by the Council on Medical Education of the American Medical Association. The program suggested in this manual will provide similar standardized training for personnel necessary to fill the key lower echelon—the laboratory assistant—who can perform many of the simpler diagnostic tests and procedures in the laboratory.

STANDARDS OF THE PROGRAM

Standards for this type of training have been established by the Board of Certified Laboratory Assistants, which was organized in 1963 by the American Society of Clinical Pathologists and the American Society of Medical Technologists. As of February 1966 the Board had approved 112 programs throughout the country. Requirements of the Board of Certified Laboratory Assistants are given in Appendix A.

In addition, the Board administers national certifying examinations for graduates of approved laboratory assistant programs. Those who pass this examination are entitled to use the designation Certified Laboratory Assistant (CLA) after their names. Such certification represents proof to a hospital or other medical laboratory that the laboratory assistant seeking employment has received adequate basic training to perform routine laboratory procedures under the direct supervision of the medical technologist and the pathologist or other qualified physician.

It is important that the training program be oriented to patient care, with competent medical control and direction. Every program should be developed within a framework that has access to the actual equipment of a laboratory as well as to a wide variety of patients providing varied clinical specimens.

From the beginning, the director of a training course for laboratory assistants should set high standards for quality performance. It is essential for students to learn a sense of responsibility toward the patient, the need to check and recheck, to ask questions when any doubt arises, and to forego the urge to take on responsibilities or decisions beyond their level of training.

This is why professional ethics require that medical technologists work under the supervision of a pathologist or other doctor of medicine, and why the laboratory assistants need to be guided and checked by the medical technologists whose broader scientific knowledge and training enable them to grasp the implications of clinical laboratory procedures and tests. In this day and age, the gap between laboratory procedures and scientific theory is not very great. A subtle difference in test results that might be observed but discounted by the scientifically uninitiated can, when fully understood, make a major difference in the handling of the patient. For this reason, it is important that laboratory personnel at each level work within their frame of scientific reference.

While educational requirements and technical skills may vary widely for the different levels, all laboratory personnel must be dedicated to a career of service in the fullest sense. Such a philosophy should be inculcated in the laboratory assistant training program, and all applicants should be screened carefully with this in mind. A sense of duty is the key to success. With it, all things are possible. Without it, the finest organizational structures are meaningless and the most excellent training may be worthless.

FUNCTIONS OF THE MEDICAL LABORATORY ASSISTANT

The medical laboratory assistant, as defined in the *Dictionary of Occupational Titles* (U.S. Department of Labor), third edition, "performs routine tests in medical laboratory for use in treatment and diagnosis of disease: Prepares tissue samples for PATHOLOGIST, takes blood samples, and prepares vaccines. Executes such laboratory tests as urinalyses and blood counts, using microscopes, micrometers, and similar instruments. Makes quantitative and qualitative chemical and biological analyses of body specimens, under supervision of MEDICAL TECHNOLOGIST or PATHOLOGIST."

Specifically, the medical laboratory assistant performs laboratory procedures in any or all of the following areas:

- *General laboratory services.* Keeps accurate records; identifies specimens properly; uses microscope and basic tools of the laboratory proficiently, including centrifuges, incubators, spectrophotometers and balances; sterilizes instruments; prepares basic laboratory solutions and media; prepares histologic and cytologic specimens for processing; and performs venipunctures and fingerpricks.

- *Bacteriology, serology, and parasitology.* Prepares and stains slides for bacteriologic study; applies sensitivity discs to culture plates and records results; prepares stool specimens for parasitologic study; performs flocculation screening serological tests for syphilis; and prepares bacteriologic and serologic specimens for mailing.

- *Hematology.* Collects and performs complete blood count, with reading of the differential count limited to normal pattern; prepares and stains blood smears; performs hemoglobin determinations; performs tests to determine bleeding time, coagulation time, sedimentation rate, and prothrombin time; and performs hematocrits.

- *Clinical chemistry.* Performs tests for nonprotein nitrogen partition (NPN and/or BUN, creatinine, uric acid); performs protein partition by a chemical method; and performs glucose, amylase, bilirubin and flocculation tests for liver function.

- *Blood bank.* Performs slide and test-tube procedures for ABO grouping and Rh typing; performs routine crossmatching and Coombs testing; prepares donor for phlebotomy; maintains blood bank records; knows how to use reference laboratories.

- *Routine analysis.* Performs routine analysis of urine, including centrifuging urine samples, preparation for microscopic study, and examination of stained and unstained sediment; titrates aspirated gastric fluid for acidity; performs test for increased globulin concentration in body fluids; performs cell count in cerebrospinal fluid; and detects occult blood and neutral fat in feces.

- *Basal metabolism and electrocardiography.* Uses and maintains machines used in these tests; prepares patient and performs tests; and detects and corrects errors.

PLACES OF EMPLOYMENT

Hospital laboratories are the largest employers of medical laboratory assistants. Opportunities also are available in public and private clinical laboratories; in physicians' offices; in public health agencies at local, State, and Federal levels; and in laboratories maintained by industrial or pharmaceutical firms.

LABORATORY STAFF ORGANIZATION AND RELATIONSHIPS

The type and extent of departmental organization in a laboratory may vary from situation to situation. If laboratory personnel are relatively homogeneous with respect to background and training, a clear-cut organizational structure may not be necessary, particularly if the laboratory is small. As the work load increases, however, the need for organization becomes more evident. Areas of activity must be clearly defined and patterns of responsibility established. Without a well-delineated administrative structure and definitive job descriptions, the duties and responsibilities of the various echelons become lost in a sea of chaotic inefficiency. Confusion and misunderstanding may develop into antagonism and insecurity. Ultimately, patient care suffers.

The director of laboratories, in most hospitals, is a physician trained in pathology. As a chief of service, his administrative position is related to the board of governors, the medical staff, and the laboratory staff. He is responsible for the total operation of the service, teaching, and research activities of the department.

Immediately below the M.D. staff comes the M.T.(ASCP) staff, that is, medical technologists certified by the Registry of Medical Technologists of the American Society of Clinical Pathologists. This staff may be divided into three echelons: chief technologist and teaching supervisor, section chief, and general duty technologist. The chief technologist and teaching supervisor usually have department head status in the hospital's organization plan, in order to deal effectively with other department heads.

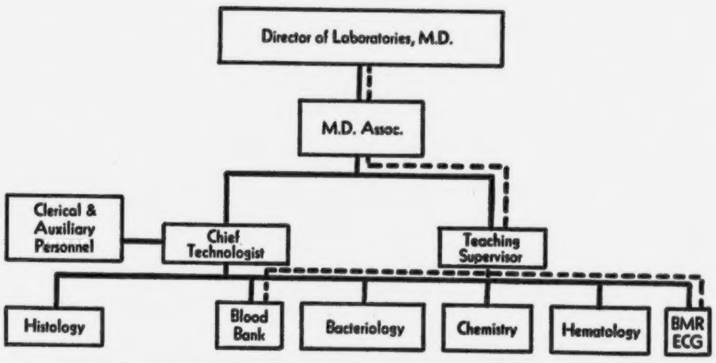
The section chief, like the head nurse, is responsible for the fiscal and technical operation of the section. Delineation of sections varies from one laboratory to another, but, in general, they consist of chemistry, hematology, blood bank, histology, bacteriology, and BMR-EKG. Serology, mycology, urinalysis, and radioisotope sections may be included under the above divisions or may constitute separate sections. If a section is sufficiently large, an assistant section chief or additional personnel may be needed.

General duty medical technologists constitute the backbone of the service force. Because of specialized knowledge and skills, the medical technologists may be utilized more efficiently and effectively by delegating many of the simpler procedures to the laboratory assistants, who come immediately below the M.T.(ASCP) staff. Laboratory assistants are specifically trained to perform these simpler procedures, with due consideration for their lack of the scientific knowledge and training needed for professional judgment in certain areas. Laboratory assistants should not be expected to participate in development, extensive quality control, or the performance of technically complicated procedures; they do not delegate responsibility nor do they supervise or teach. Their role is to perform the less complex but equally essential tasks which are necessary to complete the total service function. In the interest of good patient care it is mandatory that the tasks assigned to laboratory assistants do not exceed their capabilities, as clearly set forth in the job description.

Other technical personnel may be fitted into the organizational structure as best suits their education, experience, and training. The need of the institution for such personnel depends on such factors as size, location, specialization, and availability of other staff. Nontechnical personnel include the clerical staff, diener personnel (men of all work), and in many hospitals, an intravenous team.

ORGANIZATIONAL PLAN

The organizational plan of the laboratory is usually in writing, and appropriate changes are made from time to time as necessary. An example of such a plan is:



The solid lines represent the usual chain of command. The dotted lines represent the relationship of the teaching supervisor of the laboratory assistant training program with the section heads for the didactic and the practical aspects of the training. The chief technologist may also be the teaching supervisor.

All echelons and categories should have written job descriptions, with a full exposition of economic details, including starting salary with proper salary differentials for various categories, scale of increments, vacation and sick leave specifications, and delineation of fringe benefits such as hospitalization and retirement. A sample job description form (filled out for a laboratory assistant and excluding economic items) follows:

Job Title: *Laboratory Assistant*
Dept.: *Laboratory*

Prepared by: ....
Date: ....

Approved by: ....
(Dept. Head)
Admin.: ....

Main function: To collect blood specimens from patients and to perform certain specific laboratory procedures which are not complex in nature.

Reports to: Medical Technologist.

Duties and responsibilities: Performs the following tasks in any one or all areas (list specific tests, etc., as noted earlier under "Functions of the Medical Laboratory Assistant").

Education: High school graduation, plus completion of approved laboratory assistant training course.

Experience: Certification as Laboratory Assistant.

Contacts: Patients, nurses, physicians, and laboratory personnel.

Physical demands: Moderate to intense concentration required in using test equipment; good vision; manual dexterity.

Working conditions: Noise, odors, fumes, blood, excrement, communicable diseases; may work weekends, nights, or holidays.

Hazards: Burns from concentrated acids and alkalis; exposure to infectious diseases.

Consequences of error: Inaccurate work may invalidate test results, causing supervisors to make decisions on false information and possibly resulting in injury to patient; careless handling of chemicals and equipment may result in injury to self or others in immediate area and may destroy specimens, thus causing delay in patient's treatment and subjecting patient to additional discomfort in collecting new specimens.

PLANNING THE TRAINING PROGRAM

Training programs for laboratory assistants, who will be eligible for certification upon graduation, should be planned under the guidance of an advisory committee[1] including among its members pathologists and registered medical technologists. These programs may be established in educational institutions such as vocational and technical schools, technical institutes, 2 and 4 year colleges, and universities, in affiliation with accredited hospitals, approved medical schools or other acceptable laboratories endorsed for such training by the Board of Certified Laboratory Assistants of the American Society of Clinical Pathologists and the American Society of Medical Technologists. Or the programs may be established in these laboratories themselves.

Federal funds used to support training programs for laboratory assistants are provided by annual appropriation under the vocational education acts and the Manpower Development and Training Act of 1962. The funds are administered by the State Boards of Vocational Education.

A *Guide Book for an Approved School of Laboratory Assistants* may be obtained from the Secretary, Board of Certified Laboratory Assistants, 445 North Lake Shore Drive, Chicago, Ill., 60611. Also available is an application form for securing approval of the training program by the Board.

ORGANIZATIONAL PATTERNS

The program for laboratory assistants can use combined academic and clinical training facilities in one institution, or can be set up as separate sections in different institutions, provided that in either case the institutions involved meet minimum requirements with respect to staff and teaching facilities.

In one institution

There are several patterns that may be used for a program set up within one institution:

Concurrent Classroom and Clinical Schedules. The classroom and the practical training schedules can run concurrently throughout the period of training. The plan used to coordinate the two phases depends on the size of the institution and the staff, as well as the student load. In most instances, it will be practical to have the schedule arranged so that the student starts academic instruction in a subject before any clinical practice in that subject. Under this arrangement, the orientation period should be concentrated in the first 8 to 10 weeks of the year and be extensive enough to give the student a good introductory background in all subjects. This procedure will enable the student to understand the more basic material presented in either the classroom or the laboratory without first having had the other phase of that subject, and it will permit gradual introduction to laboratory work as the more advanced academic phases are completed.

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[1] King, Sam W., Organization and Effective Use of Advisory Committee, U.S. Office of Education, Washington: U.S. Government Printing Office, 1961.

Concurrent Schedules Using Student Laboratories. Another way to operate the academic and clinical phases concurrently would be to use student laboratories for all practical instruction. To reach the recommended minimum total of 100 class hours, at least two academic presentations would have to be given per week for 50 weeks. The students would spend the rest of the week in a student laboratory performing the practical exercises relating to the academic material. This plan presents a disadvantage in not exposing the student to the actual working conditions in a clinical laboratory.

Concentrated Academic Period Preceding Clinical Period. Classroom material can be concentrated into one period of the training year, i.e., 3 or 6 months, with the remainder of the year spent in clinical training. The concentrated academic period will be more successful if a student laboratory is available, so the student can receive practical instruction and/or demonstrations coordinated with the academic material presented.

Separate academic and clinical training

The training program can be divided into separate academic and clinical training phases, with different institutions assuming responsibility for each phase.

Academic Phase. The academic instruction can be presented in several types of institutions.

1. *Affiliation with a College or University.* An affiliation can be established between a clinical training institution and a college or university. The student might take regular college courses (usually limited to one or two basic science subjects) to give him a better background for the material to be covered in the clinical laboratory, or special courses might be set up especially to fit into the laboratory assistant's training course. In both instances, the institution giving the clinical training will have to supplement the practical work with some academic instruction. Unless the student qualifies as a regularly enrolled college student under the education laws of the State and of the college, no credit is reserved for his college hours, and they cannot be applied to future academic study.

2. *Extension or Adult Education Division of College or University.* Another type of affiliation possible is for a college or university to establish a laboratory assistant course on a noncollegiate level, as an extension or an adult education division. Under this plan, the school gives concentrated academic training to the student, usually for a 6-month period, covering all the areas of training. A student laboratory usually is provided for practical training. Instructors are medical technologists who also qualify as teachers under the staff requirements of the university or college. For their clinical training, the students are sent to affiliated medical institutions, with a member of the university or college staff acting as coordinator. College credit is not earned by the student under this program.

3. *Affiliation with Junior College.* A comparatively new type of affiliation is being established in some areas by junior colleges and clinical centers. The college and the clinical laboratory are approved jointly for a laboratory assistant course, with the college assuming responsibility for coordinating and supervising the program. The first year of the 2-year plan is spent as a full-time student in the college, with a general academic curriculum to broaden the student's educational horizons. For the second year, the student transfers to the medical laboratory for training as a laboratory assistant; the college faculty may assist the laboratory training staff in some of the academic instruction. Upon successful comple-

tion of the second year, the student is granted an associate of arts degree from the college. An incentive for the student to continue his education beyond the level of laboratory assistant is the possibility of applying credit earned during the first year toward future study in a 4-year college course.

4. *Technical or Vocational School.* The academic training can be given in an area technical or vocational school, where the teaching supervisor usually is a registered medical technologist employed by the area Board of Education. He is directly responsible for seeing that all academic instruction is presented to the student and that the practical training is provided in a student laboratory. A student-teacher ratio of 10 to 1 is approved for the academic phase of training, as contrasted with a ratio of 2 to 1 for clinical training. The academic phase is usually allotted one-half of the total time of the entire program and taught at the vocational school. However, this training can be done on hospital premises if there are better facilities and more space for instruction. In either situation, the teaching supervisor occupies the same position.

5. *Academic Facilities Provided by a Group of Hospitals.* Within one locale, it may be to the advantage of two or more hospitals or clinics to combine facilities in offering the separated academic phase of a laboratory assistant program. Selection of one of the participating institutions to be used as the center of academic training may depend on a central location, concentration of instructors, better equipment, or more adequate space. Another possibility is to divide the academic instruction into separate sections, with appropriate sections taught in separate hospitals according to their specific facilities and/or instructors available.

Clinical Phase. The clinical training period of a program providing separate academic and clinical training can be taught in a hospital laboratory or its equivalent, such as a laboratory serving a large clinic or group of doctors. One or more laboratories can be used, with the following factors guiding selection of the institutions: instructors in each section must be qualified; student-instructor ratio must not exceed 2 to 1; good facilities and equipment must be available; and the clinical material must be adequate in each specific area of training.

A student may receive all of the clinical training in one institution or in several. Careful evaluation of the clinical material and instruction available in different areas of a specific laboratory may indicate a need for rotating students among hospitals in order to achieve more comprehensive training. In such programs, the teaching supervisor for the academic instruction should be the coordinator for the clinical training, traveling between participating hospital laboratories, conducting frequent conferences with students and instructors, helping to solve problems which may arise, and maintaining school records.

If the clinical training of the entire class takes place in one institution, the teaching supervisor is needed to coordinate it with the academic training. In addition, he usually devotes some time in actual participation in the clinical training program.

STAFF COMPOSITION, QUALIFICATIONS, AND DUTIES

An adequate, interested, and well-qualified teaching staff is essential for a laboratory assistant training program. In a school where the facilities are in one institution, the desirable maximum student-teaching staff ratio is 2 to 1 for the clinical training. In other words, a school with a director, a teaching supervisor, and two instructors ideally should have no more than eight students. Because proper student instruction is time-consuming for the teaching staff, the laboratory must have adequate additional personnel for work performance. At no time should the number of students exceed the number of full-time technical workers in the laboratory.

For the school having separate facilities for the academic and the clinical training phases, a different ratio may be set up for the academic phase, with a recommended 10 to 1 maximum. However, the 2 to 1 student-instructor ratio in the clinical training phase should be maintained closely. It is felt that these recommendations will assure adequate and constant supervision in both types of programs. Students should at no time be allowed to give any type of instruction.

The teaching staff should be composed of a medical director, a teaching supervisor, and instructors.

Medical director

The medical director should be a graduate of a medical school who is certified by the American Board of Pathology or who has had acceptable training and experience in clinical pathology. The director is responsible for the training program and should be in daily attendance for a sufficient length of time to see that it is operating satisfactorily. In a program conducted by a junior college, or an area technical or vocational school, the medical director might be designated as medical coordinator or advisor.

Teaching supervisor

The teaching supervisor is the person most responsible for the success of the training program. He should be a person qualified to teach, and should have a keen interest in developing well-trained laboratory assistants.

Educational Requirements. The teaching supervisor should hold a baccalaureate degree from a recognized school of higher learning, should be a graduate of a School of Medical Technology approved by the American Medical Association, and should hold certification by the Registry of Medical Technologists of the American Society of Clinical Pathologists as M.T.(ASCP).

Clinical Experience. The teaching supervisor should have no less than three years of practical experience in the clinical laboratory, including some teaching experience and service in a supervisory capacity.

Duties. The teaching supervisor's primary responsibility is to supervise the instruction and coordinate the activities of the program. Some specific duties include:

- Reviewing applications, corresponding with applicants, and assisting in selection of students.
- Interviewing prospective students.
- Planning and scheduling classroom periods, examinations, and rotation of students.
- Teaching some of the courses and circulating constantly among the students in the various sections of the laboratory.
- Keeping permanent files and records on all students and on school activities and programs.

Instructors

Persons serving as instructors should be qualified to teach according to State certification requirements and should have an interest in the training of laboratory assistants.

Educational Requirements. Instructors should be graduates of AMA-Approved Schools of Medical Technology and registered as M.T. (ASCP), or technologists with equivalent qualifications. Specialists in the various laboratory areas, or persons possessing Master's or higher degrees, are valuable members of the staff. Information on certification requirements and availability of teacher education courses may be obtained from the State Supervisor of Trade and Industrial Education or the Head Teacher Trainer in the area.

Clinical Experience. Instructors should have at least 1 year of clinical laboratory experience.

Duties. The instructors usually present some of the classroom material and are responsible for the practical or bench work in their respective areas. Their duties include giving proper instruction in the performance of laboratory procedures; giving written, oral, and practical examinations; keeping records of tests performed; and evaluating the students' work.

Staff conferences and evaluation

The teaching staff should conduct regular conferences to compare notes on the progress of the program and problems to be solved. Complete records should be kept of these conferences, and these records should be evaluated in setting up the program for each new class of students. Periodical meetings by the director, teaching supervisor, and instructors with the students also are valuable to help evaluate what the students have learned and the teaching effectiveness of the instructors.

TRAINING FACILITIES

A well designed and equipped physical facility is basic to the effectiveness of the laboratory assistant training program. Training facilities apart from the regular laboratory should be provided for the instructional program.

Classroom

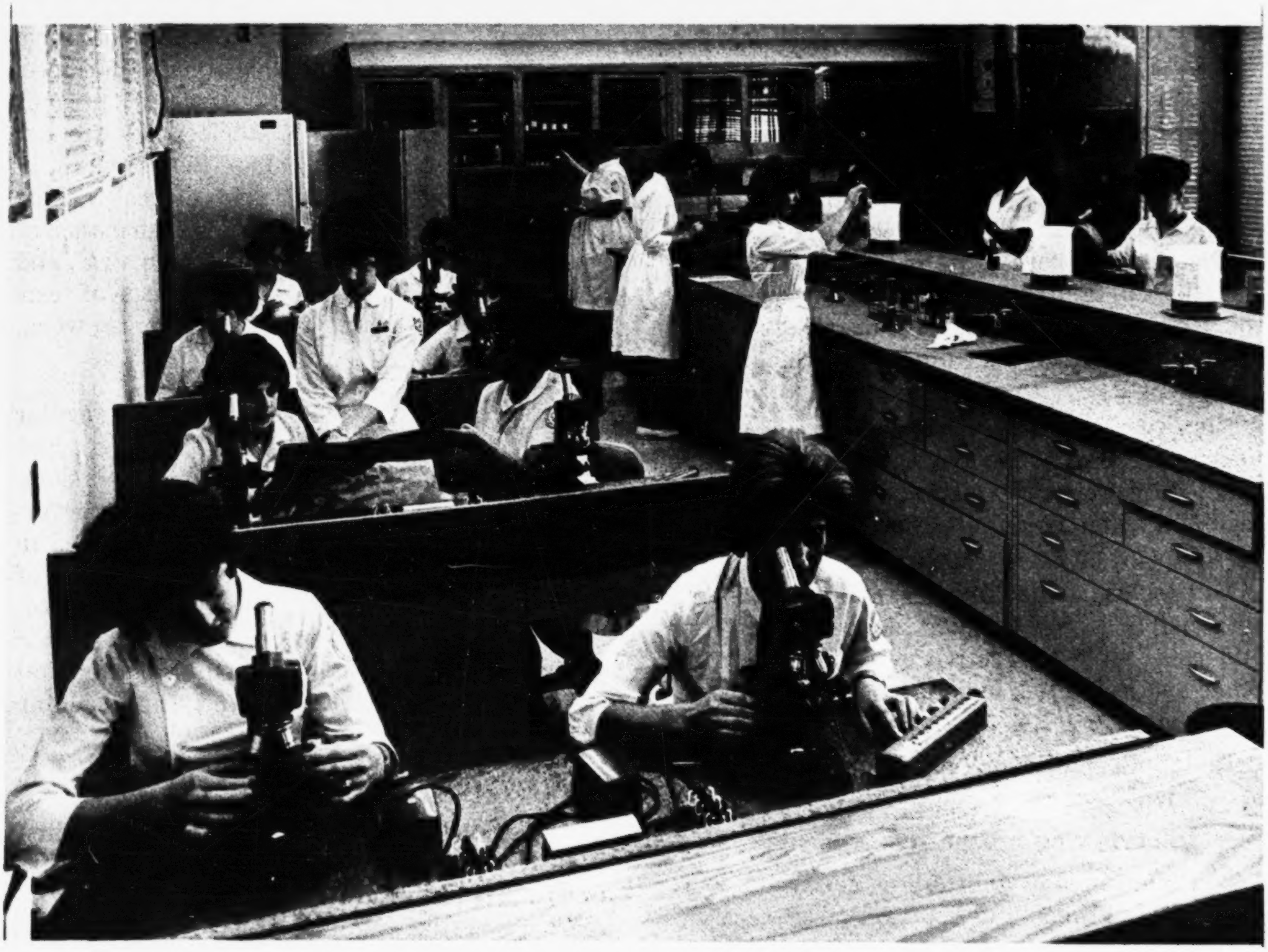
A classroom is necessary and should be available when needed. It should contain a chalkboard, ample seating and lighting, and provisions for audio-visual instruction.

Training laboratory

In a hospital training program it is advantageous to have a separate training laboratory available to provide students the opportunity for laboratory practice away from the rush of the busy hospital laboratory. A sample Layout for a Training Laboratory in a Hospital Program is shown in Appendix B. In the academic phase of a program conducted in a separate institution, a training laboratory containing all necessary equipment and facilities is essential. Appendix C shows a Sample Layout for a Training Laboratory in a Vocational School. A photograph of this laboratory, located at Schwerter School, Wichita, Kans., is shown below.

Ideally, the training laboratory should have approximately 100 square feet of space per student, including at least 3 lineal feet of workbench space per student. There should be an adequate number of electric and gas outlets, a fume hood, adequate lighting, fire-fighting equipment, and at least 1 sink per 10 students. To help determine adequate space, lighting, facilities, etc. for the laboratory, refer to "Manual for Laboratory Planning and Design," Arthur E. Rappoport, M.D., published by the College of American Pathologists, 230 North Michigan Avenue, Chicago, Ill., 60601.

Every precaution should be taken to prevent any health hazards in the laboratory. In the event any injuries occur, immediate treatment should be provided. Hospitalization insurance for the students is recommended.



Library

A library with up-to-date references, latest editions of textbooks, and scientific journals pertaining to the clinical laboratory should be maintained and readily accessible to the students.

Office and other adjacent areas

It is advantageous for the training area to have available an office for the teaching supervisor, a specimen collection or bleeding room, and restrooms or lounge.

Laboratory equipment

Equipment for a Laboratory Assistant Training Program is listed in appendix D. Suggested supplies are given in appendix E.

Clinical material

Adequate amounts and kinds of clinical material are required to assure proper practice training. There should be available material equivalent to that provided by a hospital of 100 beds, having 3,000 yearly admissions and a minimum of 50,000 clinical laboratory tests a year, with sufficient distribution of the material in order to provide adequate technical training in the various laboratory divisions. Demonstration material, including blood smears which emphasize the normal pattern, as well as color transparencies and filmstrips illustrating various techniques or test results, should be available for lectures and demonstrations.

STUDENT SELECTION

Selection of students should be based on definite criteria established by the school and made by a scholastic committee of teaching and supervisory personnel.

Educational prerequisites

Students should be graduates of accredited high schools, or have certificates of equivalent training, preferably with courses in the biological sciences, chemistry, and mathematics, including algebra and geometry. A course in elementary physics also would be helpful.

Desirable characteristics of trainees

- Good vision, needed for the delicate testing required in the use of the microscope and other sensitive optical instruments.
- Manual dexterity necessary for working with precision instruments.
- Ability to understand and follow instructions carefully and completely. (Inaccurate work may invalidate test results, and careless handling of chemicals and equipment may result in injury to self and others.)
- Ability to work with others, including patients, physicians, nurses, and other laboratory personnel.
- Integrity and intellectual honesty, with the realization that there are no shortcuts in the laboratory where tests vitally affect the lives of human beings.

Since many laboratory tests can be performed sitting down, even in a wheelchair, laboratory work offers a unique opportunity for students with physical disabilities. A number of individuals who are deaf, or who have been crippled by polio or suffer from other handicaps which do not limit their activities unduly in a laboratory situation, have discovered bright new horizons to reach for in this work. Often, government funds or grants from health agencies are available to provide vocational rehabilitation training in laboratory work for such individuals.

Admission procedure

The procedure for admitting students should include consideration of the application form with accompanying records and references, aptitude testing, and a personal interview.

Application Form. Each training program should have its own application form, with personal data such as name, address, telephone, name and address of parent or guardian, date and place of birth, marital status, educational background, past employment, military service record; possibly a brief statement of the applicant's reasons for wishing to become a laboratory assistant, and a listing of general interests, hobbies, and extracurricular activities in high school.

High School Record. A transcript of the applicant's high school record should be required, including all courses and grades, with an explanation of the school's marking system, applicant's rank in class, and scores on any specialized tests administered by the school.

Health Record. The student should submit evidence of good health through a physician's statement and report of a roentgen examination of the chest, or on a printed form included with the application form to be filled out by a physician.

Character References. Appropriate references should be obtained for all applicants. A prepared form may be sent to persons given as references, asking for opinions on specifically stated points. Most satisfactory would be references from:

- A high school science teacher, who would be queried as to the applicant's interest in science and laboratory work, his ability to follow directions and to understand basic concepts of laboratory procedures, his manual dexterity, his study habits, and an appraisal of his general intelligence.
- Former employers, youth group advisors, clergymen, or other similar sources as to the applicant's ability to get along with others, general state of health, initiative, judgment, emotional maturity, reliability, and honesty.

Aptitude and/or Interest Tests. It may be desirable to utilize aptitude testing services in the selection of students. General Aptitude Test Battery scores may be provided by the local State Employment Service. In some instances, high school transcripts may include results of aptitude and interest tests.

Personal Interview. A personal interview is an important part of selecting students. During the interview with the medical director, teaching supervisor, and possibly an instructor, judgments can best be made in regard to neatness, cleanliness, ability to communicate orally, degree of maturity, and the individual's motivation and suitability for laboratory work.

STUDENT RECORDS

A personal file should be maintained for each student, including previous scholastic record, personal references, health records, evaluations of personal factors, and, if desirable, the results of aptitude tests. The file should consist primarily of a continuing record of the student's progress through the course, with test scores, teacher evaluation, etc., from the time of application to the completion of the training program. It is valuable to continue the student's file after completion of the training in order that the school may send him relevant literature and information about new job opportunities and keep in touch with the graduate. Former students may provide the school with interesting clinical material from their laboratories for use in classroom and practice demonstrations.

Attendance records

Records of student attendance must be kept accurately day by day, so that specified hours in each phase of the course are completed. Hours lost by tardiness or absence must be made up by the end of the course.

Achievement records

Records of achievement should be kept for both phases of training—the didactic or theory, and the practical or manipulative. A student ranking at the 90th percentile in theory might possibly have to be eliminated from the course because of inability to perform the required laboratory tasks accurately and skillfully.

A conventional school roll book is used to record daily quiz grades and performance tests. A great part of the proficiency rating of the student is made through close observation by the instructor. Each examination score is recorded in the roll book, with a final grade in each section, and for the entire course.

Record of laboratory tests performed

In each learning situation, the more a student performs the various laboratory tests, the more accurate and skillful he will become. Care must be taken to record the number of tests each student has performed in each of the training areas. Equally important, the results of the work done must be recorded and compared along with standards or controls and the student graded for accuracy and precision. A sample Tally of Tests Performed is given in appendix F, and a sample Tally of Test Results in appendix G. (Details on laboratory record forms are given in appendix N.)

Rotation assignments chart

In order to plan the year's work and instructional material, the instructor must chart the course of study for the coming months. Once a satisfactory pattern is established it may be used for ensuing classes. A chart displayed for the benefit of the teacher and student not only tells the sequence of departmental study, but also keeps them both aware of the student's progress.

Evaluation and progress records

A student's progress often can be followed through periodic evaluation sheets filled out by the instruction staff. A sample of a Weekly Report of Laboratory Procedures and Assignments Completed is in Appendix H. A Monthly Report of Training is given in Appendix I, and a sample Departmental Student Report is shown in Appendix J. Some items, such as personality traits, personal hygiene, work attitude, capabilities, etc., are important for the student and staff to know. A Student Progress Report containing details of these items is shown in Appendix K. A sample of a Final Evaluation Record is given in Appendix L.

Placement file card

A program with a good method of record-keeping has at its fingertips files on all presently enrolled students, as well as past records on former students. Perspective employers may write for references on a particular graduate, and a single file card similar to the File Card on Graduates of Training Program shown in Appendix M can be used to provide the employer with the necessary information about the graduate. For example, the student might have been particularly interested and adept in chemistry or weak in some other area; the card will indicate his class standing, his final average, and any pertinent observations or comments which an instructor might otherwise forget as time passes. How a person achieves and performs in the classroom and especially in a practice laboratory is a good indication of how he will perform on the job.

DEVELOPMENT OF CURRICULUM

Instruction in the training program should follow a planned curriculum, based on the unit outlines given in the next section of this manual, including lectures, discussions, demonstrations, supervised laboratory practice, and practical clinical experience, supplemented by audio-visual aids and reading assignments.

Basic science unit

Students entering the training program may have varied backgrounds in science. An initial test may be given to evaluate their knowledge of basic chemistry. For those who need more preparation in science before starting the regular curriculum, a related Basic Science Unit in chemistry and/or biology may be offered either preceding the start of the regular program or concurrently with it. In some instances, students also may lack sufficient background in laboratory mathematics; the outline presented in the Orientation Instructional Unit for this topic may be offered at the start of the course to supplement the Basic Science Unit. This science unit can be adapted to the needs of the students; in chemistry, for example, it might include definitions and a brief outline of the following subject matter:

- Fundamental concepts of chemistry.
- Atoms and molecules.
- Valence and chemical equations.
- Oxidation and reduction.
- Physical and chemical properties of matter.
- Gases, liquids, and solids.
- Water.
- Solutions.
- Acids, bases, and salts.
- Electrolytes and ionization.
- Brief survey of organic chemistry.
- Hormones.
- Electrochemistry.

A helpful text and reference book for this basic course is *Principles of Chemistry* by Joseph H. Roe (9th ed., St. Louis, Mo., C. V. Mosby Co., 1963). Students may use only the relevant chapters.

Class schedule

In programs where the academic phase is presented for the first 6 months, the daily schedule usually includes lectures, discussions, demonstrations, laboratory practice, examinations, and field trips; the second 6 months are devoted to the clinical laboratory phase.

Where the academic and practical programs run concurrently, for the first 10 weeks of orientation, all students may follow one schedule from 8:30 a.m. to 5 p.m. During the remainder of the program, the class may operate under a two-section rotation system, with half the students starting at 8 a.m. to accompany the blood collection team and ending their day at

4:30 p.m., while the other half starts at 9 a.m. and continues on the regular schedule until 4:30 p.m., when they remain for a final hour devoted to individual review with the instructor and special projects. The two sections usually are rotated every 5 or 10 weeks. A sample daily schedule might be as follows:

8–9 a.m.	Blood collection assistance (for half the class).
9–10 a.m.	Lecture.
10–11 a.m.	Demonstration, to illustrate or augment lecture.
11–12 noon	Practice exercise.
12–12:30 p.m.	Lunch.
12:30–4:30 p.m.	Practical work in student laboratory.
4:30–5:30 p.m.	Individual review or special projects (other half of class).

Examinations

Written examinations should be given at frequent intervals, covering academic material presented in the classroom, and the solution of practical daily problems. Students in a laboratory assistant training program must assimilate a considerable amount of knowledge in a relatively short period of time. Straight recall of material in an examination is not sufficient, however. The students must have some understanding of what the knowledge means in a clinical situation in order to apply it in a working situation. Instructors interested in detailed information on preparing various kinds of items to test this type of knowledge, understanding, and application are referred to *Bulletin on Classroom Testing*, Numbers 1–12, Bureau of Institutional Research, 211 Burton Hall, University of Minnesota, Minneapolis, Minn. ($3 for entire set). Also useful are Numbers 1–3, Vol. 111, of *Teaching Tech*, edited by Verna Rausch, M.S., M.T.(ASCP), American Society of Medical Technologists, Hermann Professional Building, Houston, Tex., 77025.

Supplemental projects

In addition, supplemental projects can be useful teaching devices to augment the curriculum and add background information to help promote a sympathetic understanding among the students for other paramedical groups and their contribution to total patient care. The nature and scope of the following listed activities depend upon the imagination and ingenuity of the teaching supervisor, as well as on the level of ability and particular needs of the students. The supervisor may expand and adapt the ideas or formulate different avenues of approach consistent with local situations.

Field Trips. Suggested visits include:

- Community health centers: Board of health—special emphasis on proper preparation of bacteriology and serologic specimens for mailing; Community blood bank—preparation of donor, records, use of reference laboratory; Social welfare agencies, etc.
- Other hospitals.
- Medical exhibits, museums.
- Industrial health clinic.
- Library—orientation to catalog system.
- Hospital supply company.
- Doctor's office or clinic laboratory.
- Laboratory assistant training program in another hospital or school.
- Mental institution.

Hospital Tours. Participation of paramedical and nursing personnel in conducting inservice hospital tours provides an effective way of exposing the students to different phases of patient care. Visits may be made to:

- Dietary department—explanation of use of special diets.
- Psychiatric unit—orientation to and precautions in handling the mentally ill.
- Isolation unit—demonstration of isolation technique.
- Radioisotope laboratory—demonstration of technique.
- Intensive care unit—emphasis on emergency procedures, especially as they relate to the laboratory.
- X-rays, rehabilitation, etc.—demonstrations.
- Surgery—observation (if permitted).

Laboratory Demonstrations. These might include:

- Autopsy (optional).
- Use of specialized equipment such as automatic cell counter, flame photometer, gas analysis machine, autoanalyzer, etc.
- Gross tissue examination and tissue preparation.
- Use of fire-fighting equipment.

Student Projects. Some possible assignments might be to prepare a procedure notebook or to undertake a project related to the student's special interests.

1. *Preparation of procedure notebook.* The student is asked to prepare his own reference manual in notebook form, incorporating each new procedure to which he is introduced. The following format is used to insure inclusion of all necessary information in reference to each technique:

   Department in which test was done.
   Name of the test.
   Reagents needed.
   Chemicals necessary to make reagents.
   Chemical grades and brand names where pertinent.
   Necessary equipment for performance of test.
   Principal reaction involved.
   Procedure.
   Calculations.
   Precautions or notes.
   Controls.
   Normal values.

2. *Special interest projects.* These may be elective or assigned in addition to or in place of any of the above suggestions. They should be consistent with the student's ability and the limitations of his background. In no way should these or any other extracurricular projects be allowed to interfere with the fulfillment of the curriculum of necessary clinical experience. Examples of simple projects which may be made available to the student are:

   Work with special stains.
   Medical photography.
   Preparation of a hematology slide collection.

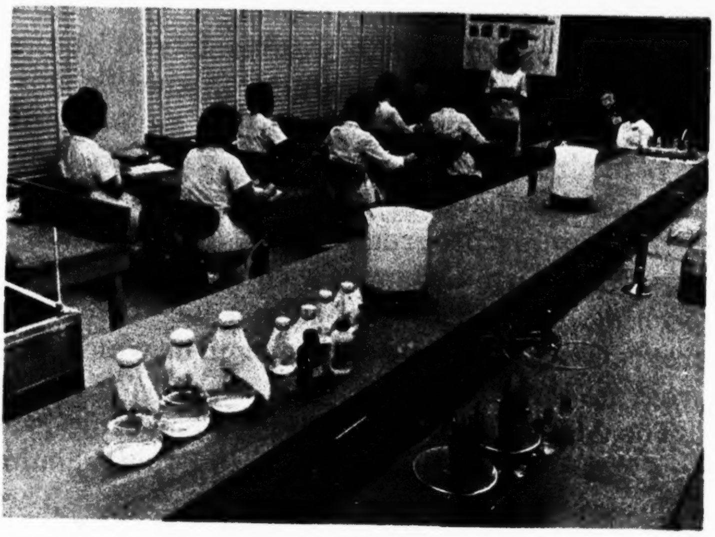
Suggested time allotments for instructional units

Hours to be scheduled for each subject may vary from school to school. Special time may be needed for supervised study, additional testing, and review, and 2 weeks usually are reserved for a vacation period. Students may need more preparation or training in one area than in another, or the teaching supervisor may find that additional classroom and demonstration time is needed to give adequate academic preparation in certain subjects. Accordingly, total hours are given, without a breakdown for classroom and practice laboratory time. The hours can be shifted to meet these needs as long as the necessary basic material is covered thoroughly and includes fundamental principles in each subject area, with special emphasis on a thorough understanding of calculations, the necessity of acquiring technical accuracy, and the limitations of this level of training.

A suggested allotment of time for the full 12 months follows:

	Weeks	*Total Hours*
Orientation to Clinical Laboratory	10	400
Bacteriology, Serology, Parasitology	8	320
Hematology	10	400
Clinical Chemistry	10	400
Blood Banking	6	240
Routine Analysis	6	240
Basal Metabolism, Electrocardiography	2	80
Total	52	2,080

In practice, most of the hours for the orientation subjects may be utilized for lectures, discussions, demonstrations, audiovisual aids, and special field trips, since few laboratory exercises are involved. The school may reduce the total time in one or more subject areas to allow for vacation periods.



INSTRUCTIONAL UNITS

Unit I Orientation to the Clinical laboratory

Suggested time: 10 weeks; 400 hours.

Introduction

The responsibility of the laboratory assistants training program to the student is twofold: first, to help the student acquire limited medical knowledge and certain skills in laboratory techniques, and second, to help him gain an understanding of the role he is to play on the laboratory team. Because of the type of student entering this program and the vast amount of material to be covered in a year's time, a detailed and thorough orientation course is necessary.

Much of the basic knowledge necessary for laboratory work will be acquired during the orientation program. The attitudes, impressions, and abilities developed by the student during the course are important and should be guided and guarded by the medical technologists responsible for his training.

The educational level of the laboratory assistant student, as well as the quantity and level of information to be acquired during the year's course, present a need for flexibility in the sequence of instruction during the orientation program. In some schools, the teaching supervisor may wish to spend 10 weeks at the beginning on orientation subjects, all didactic or possibly with some practical and laboratory-demonstration sessions. In other schools, the teaching supervisor may spend a few hours each week on introductory subjects and at the same time also initiate training in specific subject courses. This is being done in a variety of ways in already established schools. It is essential that this material be covered at some point in the training program. However, the timing can be determined most effectively by the teaching supervisor, depending on the total program schedule and the setting in which the program is taking place.

The following is not outlined by specific lecture but is an outline of material that should be presented during the training program. The placement of the material in the lecture schedule is left up to the discretion of the teaching supervisor. However, because of the nature of the first six topics, they should be presented during the first few days. The first four topics acquaint the student with his strange surroundings. The next two attempt to stimulate good study habits and professional attitudes. The remaining topics may be placed in the most appropriate positions. Several may be combined for an hour lecture. The length of time spent on each will depend on many factors—the instructor, the facilities, the interest shown by students, the availability of related institutions and facilities (state health departments, medical schools, institutional hospitals, etc.).

Demonstrations, practice work, tours, audiovisual aids, reading assignments, and special projects may be included. However, these are not detailed specifically in this section, except for a general listing of reference and textbooks, booklets, films, and filmstrips given at the end. Laboratory exercises and study questions on this orientation material would be determined by the school's own policies and procedures and could follow the relevant lecture material outlined if desired.

Unit content

A. The Scope of the Medical Laboratory Field.
B. The Training Program for Laboratory Assistants.
C. The Clinical Laboratory.
D. The Hospital.
E. Library Facilities and Suggestions for Study.
F. Medical Ethics and Conduct.
G. Introduction to Medical Terminology.
H. Laboratory Requisitions and Reports.
I. Basic Anatomy and Physiology.
J. Handling, Identification, and Care of Laboratory Equipment.
K. Aseptic Technique and Methods of Sterilization.
L. Basic Laboratory Mathematics.
M. Basic Elements of Quality Control.
N. Handling of Histologic and Cytologic Specimens.
O. Fire Safety and Disaster Planning.
P. Instruction in Blood Collection Techniques.
Q. Introduction to Departments of Clinical Pathology.

Unit outline

- A. The Scope of the Medical Laboratory Field.
  - 1. Explanation of clinical and pathological laboratory:
    - a. Role of laboratory in studying pathological changes (disease):
      - (1) Clinical laboratory findings as a diagnostic tool.
      - (2) Anatomical laboratory findings as a diagnostic and prognostic tool.
    - b. The laboratory team according to the laboratory organization chart:
      - (1) The role of the pathologist:
        - (a) Director of laboratory.
        - (b) Doctor's doctor.
        - (c) Duties and responsibilities.
        - (d) Professional organizations—American Society of Clinical Pathologists; College of American Pathologists.
      - (2) The role of the medical technologist:
        - (a) Primary and pivotal laboratory worker.
        - (b) Educational requirements, training and registration.
        - (c) Duties.
        - (d) Professional organization—American Society of Medical Technologists.
      - (3) The role of the laboratory assistant:
        - (a) Outline of program, objectives and outcomes.
        - (b) Laboratory assistant's capabilities and limitations.
        - (c) Certification by Board of Certified Laboratory Assistants as CLA (see below).
        - (d) Possible future opportunities in medical technology.
        - (e) Opportunities for further education and training.
  - 2. Medical recognition and certification:
    - a. History of Board of Registry:
      - (1) Composition:
        - (a) ASCP Representatives—five.
        - (b) ASMT Representatives—four.
      - (2) Types of certification and requirements for examination.
    - b. History of Board of Schools:
      - (1) Composition:
        - (a) ASCP Representatives—five.
        - (b) ASMT Representatives—four.
      - (2) Definition of an Approved School of Medical Technology.
    - c. History of Board of Certified Laboratory Assistants:
      - (1) Composition:
        - (a) ASCP Representatives—four.
        - (b) ASMT Representatives—four.
      - (2) Requirements for certification and approval of training programs.
- B. The Training Program for Laboratory Assistants:
  - 1. Organization and approval dates of this program:
    - a. Explanation of organizational chart of program.
    - b. Introduction of faculty members:
      - (1) Pathologist.
      - (2) Teaching supervisor.
      - (3) Instructors.
  - 2. Policies of training program:
    - a. Curriculum:
      - (1) Class schedule.
      - (2) Rotation schedule.
    - b. Fees and textbooks.
    - c. Sick leave.
    - d. Health program.
    - e. Vacation.
    - f. Stipends, scholarships, etc.
  - 3. Explanation of personnel policies of the affiliated hospital as related to students.
  - 4. Student experience records:
    - a. Distribution.
    - b. Purpose of recording experiences.
    - c. Directions for recording experiences.
  - 5. Methods of evaluation:
    - a. Class material—examinations.
    - b. Practice work—quizzes.
    - c. Personal development.
  - 6. Student notebooks:
    - a. Class notes.
    - b. Test procedures.
    - c. Special projects.
- C. The Clinical Laboratory:
  - 1. Organizational chart of laboratory.
  - 2. Scope of services:
    - a. Inpatient.
    - b. Outpatient.
    - c. Private physicians.
    - d. Charity.

    - e. Research.
  - 3. Policies and standard operating procedures of the laboratory:
    - a. Work schedule.
    - b. Work hours.
    - c. Personnel.
    - d. Vacation.
    - e. Sick leave.
    - f. Holidays.
  - 4. Use of departmental procedure manuals.
  - 5. Student manuals.
  - 6. Responsibilities of the laboratory assistant student.
- D. The Hospital:
  - 1. Hospital organizational chart.
  - 2. Interdepartmental relationships.
  - 3. Tour of hospital; introduction to department heads and explanation of services.
- E. Library Facilities and Suggestions for Study:
  - 1. Library facilities:
    - a. Clinical laboratory library.
    - b. Hospital library.
    - c. Medical school library.
    - d. Community library.
  - 2. Library orientation:
    - a. Location and hours.
    - b. Use.
    - c. Regulations:
      - (1) Card file system.
      - (2) Check-out system.
    - d. Tour of library facilities.
  - 3. Suggestions for study:
    - a. Budgeting time.
    - b. Proper conditions for study.
    - c. Good study methods.
    - d. Incentives and motivations.
    - e. Note-taking.
- F. Medical Ethics and Conduct:
  - 1. Introductory adjustments:
    - a. Professional field and ethics.
    - b. Certification examination and national recognition.
  - 2. Personal adjustments:
    - a. Personal cleanliness.
    - b. Good grooming.
    - c. The uniform.
    - d. Character—honesty, integrity, accuracy, dedication, self-control, endurance, kindness, loyalty.
    - e. Personality—manners, poise, voice, conversation.
  - 3. Patient relationship:
    - a. Specimen collection.
    - b. Answering inquiries by patients about tests and test results.
    - c. Handling special categories:
      - (1) Psychiatric patient.
      - (2) Pediatric patient; newborn infant.
      - (3) Geriatric patient.
      - (4) Uncooperative patient.
      - (5) Drunk patient.
      - (6) Patient of opposite sex.
      - (7) Comatose patient.
      - (8) Patient with communicable disease.
  - 4. Responsibilities of the laboratory assistant:
    - a. To the patient:
      - (1) Thorough understanding of orders.
      - (2) Respect for confidential matters.
      - (3) Kind and courteous treatment.
    - b. To his superiors—Medical Technologist, Pathologist, Administrator:
      - (1) Cooperation.
      - (2) Reliability.
      - (3) Respect.
  - 5. Importance of laboratory work:
    - a. Confidential nature:
      - (1) Unethical to discuss reports and findings outside the laboratory.
      - (2) Legal aspects involved in issuing laboratory results to unauthorized persons.
    - b. Accuracy and validity of laboratory results:
      - (1) Quality control.
      - (2) Care of equipment and reagents.
      - (3) Professional honesty.

- G. Introduction to Medical Terminology:
  - 1. Major Latin and Greek roots, prefixes and suffixes (given early in course).
  - 2. Abbreviations.
  - 3. Applications to terms.
  - 4. Request slip terminology. (Items 2, 3, and 4 may be given as students are exposed to them in the various departments.)

- H. Laboratory requisitions and reports:
  - 1. Types of requisition forms used in the laboratory:

- a. Admission requisition and routine laboratory tests.
- b. Requisition for other tests.
- c. Outpatient requisitions.
- d. Charge slips.

2. Identification of specimens:
   - a. Information on requisition slips.
   - b. Information on label of container.
   - c. Method of listing and numbering of specimens:
     - (1) Transported from laboratory to other institutions.
     - (2) Received into laboratory:
       - (a) Inpatient.
       - (b) Outpatient.
       - (c) Mail.
   - d. Charting test results:
     - (1) Explanation of patient's chart.
     - (2) Methods of charting test results.
   - e. Explanation of filing system within laboratory.
   - f. Tabulation of test performed:
     - (1) Daily records.
     - (2) Monthly records.
     - (3) Yearly records.
   - g. Processing reports on "Stat" work.

I. Basic Anatomy and Physiology:

A brief description and discussion of the various systems of the human body, their characteristics and functions, will be necessary to acquaint the student with the body as a whole. More detailed discussions can be given in the various departments as part of the introduction to the specific subject matter.

1. Introduction:
   - a. Major concepts about the body.
   - b. Component units of the body.
2. The erect and moving body:
   - a. The skeletal system.
   - b. The muscular system.
3. Maintaining the metabolism of the body:
   - a. The respiratory system.
   - b. The digestive system.
   - c. The circulatory system.
   - d. The urinary system.
4. Integration and control of the body—the nervous system.
5. Integration and control of body functions by hormones—the endocrine system.
6. Reproduction of the human being—the reproductive system.

J. Handling Identification, and Care of Laboratory Equipment:

1. General considerations:
   - a. Precautions in handling.
   - b. Breakage and repayment.
   - c. Expense and replacement cost.
2. Glassware:
   - a. General considerations:
     - (1) Kinds of glass.
     - (2) Maintenance of glassware supplies.
     - (3) Location of storage areas.
     - (4) Cleaning of glassware:
       - (a) Methods used in laboratory.
       - (b) Cleaning of specific items.
       - (c) Importance of thorough rinsing.
       - (d) Spot-checking for proper cleaning.
     - (5) Plastic items versus glassware:
       - (a) Advantages and disadvantages.
       - (b) Cost.
   - b. Care of needles and syringes:
     - (1) Cleaning.
     - (2) Sterilization.
3. The Microscope:
   - a. The compound microscope:
     - (1) Review of parts.
     - (2) Theory of the microscope.
     - (3) Use of the microscope.
     - (4) Care of the microscope.
   - b. Special microscopes:
     - (1) Darkfield.
     - (2) Stereo.
     - (3) Electron.
     - (4) Phase.
   - c. Demonstration and practice in use of microscope.
4. The Balance:
   - a. Introduction to the analytical balance:
     - (1) Construction and principal parts.
     - (2) Care in cleaning.
     - (3) Proper operation and rules.
     - (4) Weights:
       - (a) Use and care of weights.
       - (b) Periodic check on weights.
   - b. Other balances—description and uses:
     - (1) Torsion.
     - (2) Trip.
   - c. Laboratory exercise in use of balances.

5. Electrical instruments:
   - a. Centrifuges.
   - b. Rotators.
   - c. Waterbaths.
   - d. Incubators.
   - e. Routine care:
     - (1) Cleaning and oiling.
     - (2) Maintenance.
     - (3) Responsibility of personnel.
   - f. Instrument failure.
   - g. Demonstration and practice in use of instruments.
6. Electronic instruments:
   - a. Introduction to instruments.
   - b. Instruction manuals.
   - c. Responsibility of personnel toward instrument.
   - d. Replacement of parts.
   - e. Instrument failure.
   - f. Proficiency in use.
   - g. Demonstration and practice in use of instruments.

K. Aseptic Technique and Methods of Sterilization:

1. Principles of aseptic technique. (Some of this material also is covered in serology; it may be omitted at that time or repeated as a review.):
   - a. Definition of asepsis.
   - b. Requirements and utilization of sterile technique.
   - c. Materials and equipment used:
     - (1) Syringes:
       - (a) Types and sizes.
       - (b) Reusable versus disposable—advantages and disadvantages.
     - (2) Needles:
       - (a) Types and sizes.
       - (b) Reusable versus disposable—advantages and disadvantages.
     - (3) Vacutainers:
       - (a) Types and sizes.
       - (b) Anticoagulants.
       - (c) Advantages and disadvantages.
   - d. Performing a venipuncture:
     - (1) Preparation of patient.
     - (2) Technique:
       - (a) Use of syringe.
       - (b) Use of vacutainer.
   - e. Opening an ampule.
   - f. Care of soiled supplies and equipment.
2. Precautions to take upon entering:
   - a. Operating room.
   - b. Delivery room.
   - c. Nursery.
   - d. Isolation.
3. Methods of sterilization:
   - a. Heat:
     - (1) Temperature.
     - (2) Time.
     - (3) Moisture.
     - (4) Pressure.
   - b. Chemical.
   - c. Filtration.
   - d. Checking the sterilization procedure.

L. Basic Laboratory Mathematics:

1. Review of student weakness:
   - a. Fractions.
   - b. Decimals.
   - c. Percentages.
   - d. Ratio and proportion.
2. Metric system:
   - a. Linear measure.
   - b. Volume.
   - c. Weight.
   - d. Conversion problems.
3. Importance of accurate glassware:
   - a. Bureau of Standards.
   - b. Necessity for calibrated glassware.
   - c. Varieties of pipettes, tubes, flasks.
   - d. Precision in pipetting.
4. Solutions:
   - a. Types of solutions:
     - (1) Normal.
     - (2) Percent.
     - (3) Molar.
     - (4) Weight/volume.
     - (5) Volume/volume.
     - (6) Weight/weight.
     - (7) Aqueous, alcoholic, etc.
   - b. Solution problems.
   - c. Preparation of solutions.
5. Titrations:
   - a. pH, definition.
   - b. Selection of indicators.
   - c. Preparation and titration of solutions.
6. Miscellaneous measurements and calculations:
   - a. Temperature and heat:
     - (1) Temperature scales:
       - (a) Fahrenheit.
       - (b) Centigrade.

- (c) Formulas for conversion.
  - (2) Quantities of heat.
  - (3) Bunsen burner.
- b. Calculations—common calculations in the laboratory.

7. Review of basic chemistry mathematics:
   - a. Valance and chemical equations.
   - b. Atoms and molecules.
   - c. Oxidation and reduction.
   - d. Acids and bases.
   - e. Normality, molarity, milliequivalents.

M. Basic Elements of Quality Control:

1. Meaning of quality control:
   - a. Concept of positive and negative controls.
   - b. Standard solutions.
   - c. Concept of variance and standard deviation.
2. Procedures to minimize errors:
   - a. Correct identification of patient.
   - b. Obtaining representative and adequate specimens.
   - c. Correct identification of specimens.
   - d. Accurate analysis of specimens.
   - e. Prompt and accurate reporting of results.
   - f. Adequate recordkeeping.
3. Types of control measures taken in various departments of the laboratory.
4. Moral responsibility of student in this area, as related to:
   - a. Patient.
   - b. Technologist.
   - c. Physician.
   - d. Self.

N. Handling of Histologic, Cytologic, and other Specimens:

1. Introduction to histopathology:
   - a. Role of histopathology in modern medicine:
     - (1) The biopsy.
     - (2) The autopsy.
   - b. The histology laboratory and its relationship to other departments of the laboratory.
   - c. Safety precautions in handling specimens.
   - d. Preparation of tissue specimens for mailing.
   - e. Recordkeeping.
2. Introduction of cytology:
   - a. Role of cytology in modern medicine:
     - (1) Definition.
     - (2) History.
     - (3) Papanicolaou smear.
   - b. The cytology laboratory and its relationship to other departments of the laboratory.
   - c. Safety precautions in handling specimens.
   - d. Preparation of specimens for mailing.
   - e. Recordkeeping.
3. Mailing of specimens to larger or reference laboratories:
   - a. Collection of specimens:
     - (1) Blood smears.
     - (2) Bacteriological.
     - (3) Parasitological.
     - (4) Serological.
   - b. Handling of specimens.
   - c. Containers for mailing.
   - d. Preparation.

O. Fire Safety and Disaster Planning:

1. Safety program of the hospital:
   - a. Fire plan:
     - (1) Fire signal.
     - (2) Location of fire boxes.
     - (3) Location, use, and maintenance of fire-fighting equipment.
     - (4) Station and duties of the laboratory personnel in emergency.
   - b. Disaster plan:
     - (1) Warning signal.
     - (2) Location of shelter.
     - (3) Station and duties of laboratory personnel in case of disaster.
2. Laboratory safety and first aid:
   - a. Precautions to be used in:
     - (1) Collection of specimens.
     - (2) Receipt of specimens.
     - (3) Handling of specimens.
     - (4) Disposing of glass, flammable materials, contaminated materials, etc.
   - b. Basic precautions to be observed for control of chemical hazards, explosives, etc.
   - c. Safety practices in electrical maintenance and operation.
   - d. Safety practices in handling other laboratory equipment — especially glassware.

- e. Reporting of accidents.
- f. Laboratory first aid and fire-fighting:
  - (1) Safety equipment available in laboratory:
    - (a) First aid kit or cabinet.
    - (b) Safety shower and eye fountain.
    - (c) Caution labels.
    - (d) Tongs for handling hot utensils.
    - (e) Protective coverings: coats and aprons; gloves and mittens.
  - (2) Fire-fighting methods and equipment for the laboratory.
  - (3) Use of fume hood.
  - (4) Care of sinks and drains.

P. Instruction in Blood Collection Techniques. (Some of this material may be covered under Blood Banking and/or Serology or repeated there for review.):

- 1. Hospital regulations concerning collection of specimens.
- 2. Review of blood-carrying organs of the body.
- 3. Collection of blood:
  - a. Type of specimen requested:
    - (1) Serum.
    - (2) Whole blood.
    - (3) Plasma.
  - b. Use of anticoagulants.
  - c. Blood for chemical analysis:
    - (1) Containers.
    - (2) Preparation of the patient.
    - (3) Time of collection.
    - (4) Type of specimen.
    - (5) Amount of blood required; use chart.
  - d. Blood for serologic tests (including blood banking): give same information as in c—(1) to (5).
  - e. Blood for hematologic tests: give same information as in c—(1) to (5).
  - f. Blood for microbiologic examinations: give same information as in c—(1) to (5).
- 4. The Venipuncture:
  - a. Preparation of utensils needed.
  - b. Preparation of skin.
  - c. Selection of most suitable vein.
  - d. Technique of venipuncture.
  - e. Failure to obtain blood:
    - (1) Causes.
    - (2) Remedies.
  - f. Precautions to avoid errors, hemolysis, etc.
  - g. Demonstration and practice sessions in performing venipuncture.
- 5. Collection of capillary blood:
  - a. Materials for collection tray.
  - b. Site of collection.
  - c. Steps in collection.
  - d. Precautions to avoid errors.
  - e. Demonstration and practice sessions in performing puncture.

Q. Introduction to Departments of Clinical Pathology:

- 1. Bacteriology, serology, and parasitology:
  - a. Bacteriology:
    - (1) Branches of microbiology:
      - (a) Bacteriology.
      - (b) Mycology.
      - (c) Virology.
    - (2) History of bacteriology.
    - (3) Morphology and physiology of bacteria.
  - b. Serology:
    - (1) Use of serological tests in diseases other than syphilis.
    - (2) Growth of serology and immunology.
  - c. Parasitology:
    - (1) Difference between animal parasites and bacteria.
    - (2) Relationship of parasites and vectors.
  - d. Interrelationship of bacteriology, serology, and parasitology.
- 2. Hematology:
  - a. Function of blood in health and disease.
  - b. Its relationship to clinical diagnosis and treatment.
- 3. Chemistry:
  - a. Fundamental concepts.
  - b. Introduction to and instruction in use of instruments used in chemical analysis.
- 4. Blood banking:
  - a. Responsibilities and limitations of laboratory assistant in this field.
  - b. Problems in blood banking:
    - (1) Legal.
    - (2) Technical.
  - c. Brief history of blood banking.
  - d. Basic blood groups.

5. Routine analysis:
   a. Urinalysis:
      (1) Importance of urinalysis as study of end product of metabolism.
      (2) Kidney function in health and disease.
   b. Gastric analysis:
      (1) Introduction.
      (2) Titration of acidity.
   c. Cerebrospinal fluid—cell count.
   d. Occult blood and neutral fat in feces.
6. Basal metabolism and electrocardiograph:
   a. BMR—introduction and purpose.
   b. EKG—introduction and purpose.

Bibliography and Audiovisual Aids

A great part of the material to be included in the orientation lectures and discussions can be found in the textbooks, references, journals, booklets, and audiovisual aids listed below. Students should be required to read assignments pertaining to subjects covered during the orientation period in the textbooks. *An introduction to the Study of Disease* by Dr. William Boyd, in particular, contains much useful basic information on these topics, and other assignments might be made from this text to supplement material covered in other instructional units. The booklets and leaflets listed should be assigned to the students for study, as should the laboratory's administrative manual, the ward manual, and other materials related to the functions of the clinical laboratory in which the students will receive their practical instruction.

A wide variety of films and filmstrips is available on the topics included in the Orientation program, as well as on the various subject units. Those listed are considered most useful at the laboratory assistant level. A suitable selection may be made for showing to the students to supplement orientation classes and/or during the specific subject unit periods. Some may be indicated again under unit outlines because of their special relevancy to that particular topic.

Basic textbooks

Anthony, Catherine, *Textbook of Anatomy and Physiology*, 5th ed., St. Louis, Mo., C. V. Mosby Co., 1959.

Boyd, William, *An Introduction to the Study of Disease*, 5th ed., Philadelphia, Lea and Febiger, 1962.

Davidsohn, I., and Wells, B. B., *Clinical Diagnosis by Laboratory Methods*, 13th ed., Philadelphia, W. B. Saunders, 1962.

Goss, Charles Mayo, *Gray's Anatomy of the Human Body*, 27th ed., Philadelphia, Lea and Febiger, 1959.

Kiely, L. J., and Langley, L. L., *Wookbook and Laboratory Manual on Human Anatomy and Physiology*, New York, McGraw Hill Co., 1963.

Lynch, M. J., Raphael, S. S., and others. *Medical Laboratory Technology*, Philadelphia, W. B. Saunders, 1963.

MacFate, R. P., *Introduction to the Clinical Laboratory*, Chicago, Year Book Medical Publishers, Inc., 1961.

Roe, J. H., *Principles of Chemistry*, 9th ed., St. Louis, Mo., C. V. Mosby. 1963.

References

Board of Certified Laboratory Assistants, *Guide Book for an Approved School of Laboratory Assistants*, Secretary of the Board, 445 North Lake Shore Drive, Chicago, 1963.

Cook, E. B. M., "Safety in the Public Health Laboratory," *Public Health Reports*, 76:51, January 1961.

Dorland, W. A. *American Illustrated Medical Dictionary*, 23d ed., Philadelphia, W. B. Saunders, 1958.

Ederer, Grace Mary, and Tucker, Barbara, "Accident Survey and Safety Programs in Two Hospital Clinical Laboratories," *American Journal of Medical Technology*, 26:219-227, July–August 1960.

Freney, Sister Mary Agnes Claire, *Understanding Medical Terminology*, St. Louis, Mo., Catholic Hospital Association, 1958.

Montgomery, Lall, G., "The Work of the Registry of Medical Technologists," *American Journal of Medical Technology*, 25: 295-303, September–October 1959.

Morrison, John, "The Medical Laboratory Technologist as a Professional Member of the Patient's Team," *American Journal of Medical Technology*, 26:252-256, July–August, 1960.

Rausch, Verna, Hovde, Ruth, and Ohlen, Margaret, "A Training Program for Medical Laboratory Assistants," *American Journal of Medical Technology,* 25:287–328, September–October 1959.

Smith, Harry P., "Legal Responsibilities and Liabilities of Pathologists and Medical Technologist," *American Journal of Medical Technology,* 26:11–18 January–February 1960.

Booklets and leaflets for students

Blood Groups, chart, Scientific Products Co., Evanston, Ill.

Care and Handling of Glass Volumetric Apparatus, Kimble Co., Toledo, Ohio.

Common Medical Terminology, Abbott Laboratories, North Chicago, Ill.

How to Care for Hypodermic Syringes and Allied Products, Becton, Dickinson and Co., Rutherford, N.J.

Introduction to Biological Latin and Greek, by Patrick H. Yancey, Spring Hill College Press, Mobile, Ala. 1959.

Manual of Laboratory Safety, Fischer Scientific Co., Pittsburgh, Pa.

Terms Used in Microscopy, American Optical Co., Instrument Division., Buffalo, N.Y.

The Theory of the Microscope, Bausch and Lomb Optical Co., Rochester, N.Y.

The Use of Blood, Abbott Laboratories, North Chicago, Ill.

Films (all 16 mm.).

A Control Program for Cross Infection in a Modern Hospital, color, sound, 22 min., Winthrop Laboratories, New York, N.Y.

Analytical Balance and Its Use, B. & W., sound, 16 min., Fischer Scientific Co., Pittsburgh, Pa.

Chemical Lab Safety, color, sound, 25 min., Communicable Disease Center, Atlanta, Ga.

From One Cell, color, sound, 15 min., American Cancer Society local chapters.

Hemo—The Magnificent, color, sound, 59 min., Bell Telephone business offices.

Hospital Care of Syringes and Needles, Part II, color, sound, 20 min., Becton, Dickinson and Co., Rutherford, N.J.

Hospital Sepsis: A Communicable Disease, color, sound, 30 min., Hospital Division, Johnson and Johnson, New Brunswick, N.J.

Infectious Diseases and Man-made Defenses, color, sound, 11 min., Coronet Films, Chicago, Ill.

Modern Techniques of Collecting Blood Samples, color, sound, 44 min., Becton, Dickinson and Co., Rutherford, N.J.

No Margin for Error, B. & W., sound, 30 min., Motion Picture Library, American Medical Association, Chicago, Ill.

Safety in the Chemistry Laboratory, B. & W., sound, 15 min., Audio-Visual Extension, University of Minnesota, Minneapolis, Minn.

Sterilization Procedures in the Medical Office, color, sound, 29 min., Wyeth Film Library, Box 8299, Philadelphia, Pa.

The Compound Microscope, color, sound, 20 min., Bausch and Lomb Optical Co., Rochester, N.Y.

The Nature of Glass, color, sound, 37 min., Association Films, La Grange, Ill.

Vacutainer Technique, color, sound, 50 min., Becton, Dickinson and Co., Rutherford, N.J.

Venipuncture, color, sound, 15 min., Imperial Chemical Industries, New York, N.Y.

Weighing with the Analytical Balance, B. & W., sound, 20 min., University of Minnesota, Minneapolis, Minn.

Why Johnny Bleeds, color, sound, 18½ min., Medical Illustration Service, Armed Forces Institute of Pathology, Washington, D.C.

William Harvey and the Circulation of the Blood, color, sound, 36 min., American Medical Association, Chicago, Ill.

Work of the Blood, color, sound, 13 min., Encyclopaedia Britannica Films, Wilmette, Ill.

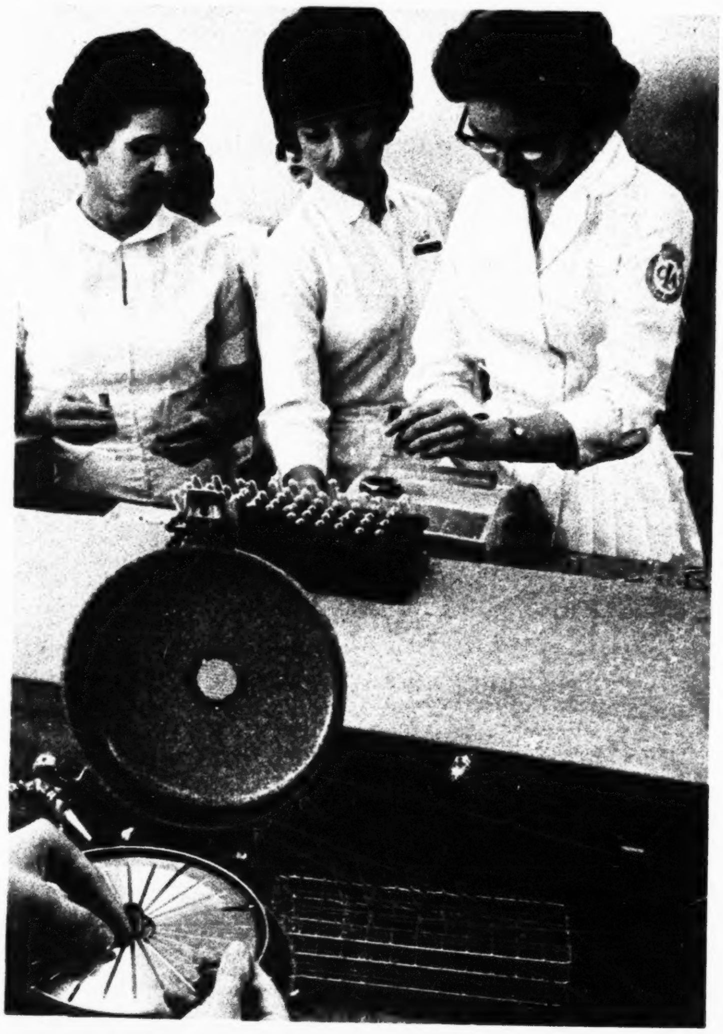
Filmstrips

Acid and Basic Solutions, B. & W., 10 min., McGraw-Hill Film Dept., New York, N.Y.

Anatomy and Physiology, filmstrip series on digestive, endocrine, urinary, and other body systems, 35 min., color, with record, U.S. Army (available from Central Film Exchanges at most area Army Headquarters).

Handling and Use of Glassware, color, sound, 8 min., Communicable Disease Center, Atlanta, Ga.

(Note.—Numerous other films and filmstrips are available from pharmaceutical companies, health organizations, government agencies, educational institutions, etc. Film catalogs can be obtained by the teaching supervisor from these and other sources; a useful guide is "Film Reference Guide for Medicine and Allied Sciences," U.S. Government Printing Office, Washington, D.C. Audiovisual Seminar filmstrips of the Commission on Continuing Education, American Society of Clinical Pathologists, 445 North Lake Shore Drive, Chicago, Ill., 60611, may be too technical for this level, but can be stopped several times for more detailed explanations to simplify the material. Bulletins issued three times a year by the Audio-Visual Library of the ASMT Education & Research Fund, Inc., Suite 25, Hermann Professional Building, Houston, Tex., 77025, evaluate films, filmstrips, and other educational materials; the Library also has a number of films and filmstrips available on loan.)



Unit II Bacteriology

Suggested time: 8 weeks; 320 hours (This is the period suggested for the combined courses of Bacteriology, Serology, and Parasitology covered in Units II, III, and IV.)

Introduction

The outline of this course, and the succeeding two on Serology, Parasitology, includes instructional aids and guidance in only the elementary and basic skills encountered in the microbiology laboratory. Basic principles of bacteriology, serology, and parasitology are covered in the Orientation Section. The student should be allowed sufficient time to practice the skills described in the course so that he may attain a satisfactory level of performance.

It is suggested that the student use a notebook for the laboratory exercises as well as for class notes. All drawings, lists, descriptions of test procedures, and outlines kept in such a manner may be readily available as reference material.

Unit content

A. Preparation of Glassware, Pipettes, and Test Tubes for Sterilization.
B. Sterilization of Glassware and Media.
C. Preparation and Storage of Media.
D. Preparation of Smears; Sensitivity Studies.
E. Preparation of Stains and Staining Techniques.
F. Receiving, Handling, Processing, and Disposal of Infectious Materials.
G. Preparation of Bacteriological and Serological Specimens for Shipment.

Bibliography

Bailey, W. R., and Scott, E. G., *Diagnostic Microbiology,* St. Louis, Mo., C. V. Mosby Co., 1962.

Burrows, William, *Textbook of Microbiology,* 17th ed., Philadelphia, W. B. Saunders, 1957.

Dubos, Rene, ed., *Bacterial and Mycotic Infections of Man,* 4th ed., Philadelphia, J. B. Lippincott, 1964.

Hepler, O. E., *Manual of Clinical Laboratory Methods,* 4th ed., 13th Printing Springfield, Ill., Charles C. Thomas, 1963.

Levinson, S. A., and MacFate, R. P., *Clinical Laboratory Diagnosis,* 6th ed., Philadelphia, Lea and Febiger, 1961.

Perkins, John J., *Principles and Methods of Sterilization,* Springfield, Ill., Charles C. Thomas, 1960.

Schaub, I. G., Foley, T., and others, *Diagnostic Bacteriology,* 5th ed., St. Louis, Mo., C. V. Mosby Co., 1958.

Simmons, J. S., and Gentzkow, C. J., *Medical and Public Health Laboratory Methods,* Philadelphia, Lea and Febiger, 1956.

Smith, D. T., Conant, N. F., and Overman, J. R., *Zinsser's Bacteriology,* 13th ed., New York, Appleton-Century-Crofts, Inc., 1964.

Stewart, F. S., *Bigger's Handbook of Bacteriology;* 7th ed., Baltimore, Md., Williams and Wilkins Co., 1959.

Manuals

B. B. L. *Products Listing,* B. B. L., Inc., 2201 Aisquith St., Baltimore 18, Md., 1956.

Catholic Hospital Association, *Newer Concepts and Techniques in Clinical Diagnostic Microbiology,* St. Louis, Mo., the Association, 1959.

Department of the Air Force, *Laboratory Procedures in Clinical Bacteriology,* Air Force Manual, U.S. Government Printing Office, Washington, 1962.

Difco Laboratories, *Difco Manual,* 9th ed., Detroit, Michigan, The Laboratories, 1953.

Kimler, Alexander, *Manual of Clinical Bacteriology,* Philadelphia, J. B. Lippincott, Co., 1961.

Unit outline

A. Preparation of Glassware, Pipettes, and Test Tubes for Sterilization:
  1. Types of glassware used in the bacteriology laboratory:
    a. Flasks, graduates, funnels, bottles.
    b. Glass containers used for the collection of specimens.
    c. Petri dishes, Spray or Bray dish, Brewer anaerobic dish.
    d. Glass slides.
    e. Glass streaking rods.
    f. Syringes.
  2. Types of pipettes used in the bacteriology laboratory:
    a. Serologic, volumetric.
    b. Automatic pipettors.
  3. Types of test tubes used in the bacteriology laboratory:
    a. Bacteriological.
    b. Screw cap.
    c. Dunham (gas fermentation).
    d. Others.
  4. Cleaning, plugging, and wrapping of glassware:
    a. Flasks, graduates, funnels.
    b. Bottles.
    c. Petri dishes.
    d. Glass slides.
    e. Streaking rods.
    f. Pipettes.
    g. Test tubes.
  5. Disposable plastic equipment:
    a. Petri dishes.
    b. Pipettes.
    c. Test tubes and applicators.
    d. Vials.
  6. Use of metal containers for sterilizing:
    a. Petri dishes.
    b. Pipettes.

Suggested reading assignments for students

Air Force Manual, Chapter 1; pp. 17–23.
Bailey and Scott, Chapters 6 and 7.
Simmons and Gentzkow, pp. 617–618.

Audiovisual Aids

Chemical Techniques, series of 3–5 minute color, sound films of Volumetric Flasks, Special Pipettes, and Pipettors, Naval Medical School, Audiovisual Division, Building 141, Room 11-B, Bethesda 14, Md.

Laboratory Exercises

1. In your notebook, draw, label, and name the different types of flasks, graduates,

funnels, and bottles that are used in the bacteriology laboratory.

2. In your notebook, draw the different types of glass containers that are used for the collection of specimens. Label each and indicate the purpose for which it is used.
3. In your notebook, draw, label, and name the different types of pipettes and test tubes that are used in the bacteriology laboratory.
4. Practice making cotton plugs for flasks, test tubes, and pipettes.
5. Practice preparing syringes, flasks, test tubes, and pipettes for sterilization.
6. Practice preparing glass slides for sterilization.

Study Questions

1. What is the difference between a Florence and an Erlenmeyer flask?
2. What type of container would be used to collect the following specimens:
   a. 24 hr. urine culture.
   b. Sputum.
   c. Nose and throat culture.
   d. Stool culture.
   e. Blood culture.
3. Why is it necessary that the pipettes used in the bacteriology laboratory be plugged?
4. For what purpose are the following pieces of glassware used:
   a. Petri dish.
   b. Spray dish.
   c. Streaking rod.
   d. Syringe.
   e. Gas fermentation tube.
5. How is cotton prepared or folded for plugging flasks?
6. For what kind of equipment may metal containers be used for sterilization?.
7. What procedure is followed for cleaning the following:
   a. Petri dishes.
   b. Pipettes.
   c. Flasks.
   d. Slides.
   e. Test tubes.
   f. Syringes.
8. What effect would moisture on cotton plugs have on the sterility of media?

B. Sterilization of Glassware and Media:

1. Methods of sterilization—dry heat:
   a. Temperature.
   b. Exposure time.
   c. Types of glassware that can be sterilized by this method.
2. Methods of sterilization—moist heat:
   a. Autoclave and Arnold sterilizer.
   b. Method of operation.
   c. Temperature.
   d. Pressure.
   e. Exposure time.
   f. Types of glassware and media that can be sterilized by this method.
3. Methods of sterilization—boiling:
   a. Temperature.
   b. Exposure time.
   c. Types of glassware and media that can be sterilized by this method.
4. Methods of sterilization—filtration:
   a. Types of filters.
   b. Media that can be sterilized by this method.
5. Methods of checking the effectiveness of autoclave:
   a. Recording thermometers.
   b. Sterilization indicators.

Suggested reading assignments for students

Bailey and Scott, Chapter 1

Audiovisual Aids

Pressure Steam Sterilization, color sound, 30 min., Dr. Harry E. Morton, University of Pennsylvania School of Medicine, Philadelphia 4, Pa.

Laboratory Exercises

1. In your notebook, make a list of glassware that can be sterilized by:
   a. Dry heat.
   b. Moist heat.
   c. Boiling.
2. In your notebook, make a list of media that can be sterilized by:
   a. Moist heat (autoclave).
   b. Moist heat (Arnold sterilizer).
   c. Boiling.
   d. Filtration.
3. In your notebook, draw a picture of an

autoclave and label the following parts:
  - a. Safety door.
  - b. Condensation shield.
  - c. Pressure gauge.
  - d. Safety valve.
  - e. Exhaust valve.
  - f. Thermometer.
  - g. By-pass valve.
  - h. Discharge valve.
  - i. Condensation exhaust.
4. Practice sterilization of glassware and media using the autoclave.

Study Questions

1. When would fractional sterilization be employed?
2. At what temperature, pressure, and length of exposure time is routine media sterilized in the autoclave?
3. When would filtration be employed as a means of sterilization?
4. At what temperature and exposure time should glassware be sterilized in the hot air oven?
5. Name three types of filters that may be used in filtration sterilization.
6. What methods may be used to test the efficiency of the autoclave?

C. Preparation and Storage of Media:

1. Physical states of media:
   - a. Solid (agars).
   - b. Semi-solid (gelatins).
   - c. Liquid (broths).
2. Weighing media:
   - a. Types of balances.
   - b. Balancing of scale.
   - c. Measurement of media.
3. Dilution and heating:
   - a. Use of distilled water.
   - b. Technique in heating media.
4. Adjustment of pH by Colorimetric method.
5. Dispensing media prior to sterilization for:
   - a. Broth—tubes and flasks.
   - b. Agar—tubes and flasks.
   - c. Dunham fermentation tubes.
6. After sterilization the technique of making:
   - a. Agar plates.
   - b. Agar slants.
   - c. Blood agar plates.
   - d. Chocolate agar plates.
7. Storage of media:
   - a. Refrigeration.
   - b. Room temperature.

Suggested reading assignments for students

BBL Products Manual, General suggestions, pp. 1–2.
Bailey and Scott, Chapter 1; pp. 19, 20, and 24.
Difco Manual, pp. 21–22.
Kimler, pp. 9, 11.
Simmons and Gentzkow, pp. 630–631.
Stewart, pp. 35–43.

Laboratory Exercises

1. Practice weighing, heating, dispensing, and sterilizing the following:
   - a. Broth.
   - b. Basic agar for: plates agar slants.
2. Practice making the following:
   - a. Blood agar plates.
   - b. Citrate agar slants.
   - c. Urea agar slants.
   - d. Bordet-Gengou agar plates.
3. Practice making the following:
   - a. Chocolate agar plates.
   - b. Gas fermentation (Dunham) tubes.
   - c. Bismuth sulfite plates.
   - d. Selenite broth.
   - e. Litmus milk.
4. In your notebook, make a list of the media used in your laboratory that should not be autoclaved.
5. In your notebook, make a list of the media used in your laboratory that should be slanted.
6. In your notebook, make a list of the media used in your laboratory that should be put into Petri dishes.

Study Questions

1. What type of media should be stored in the refrigerator?
2. Describe the technique used for making blood plates.
3. What do the following abbreviations stand for:
   - a. T.S.I. agar.

- b. E.M.B. agar.
- c. B.C. agar.
- d. S.S. agar.

4. What percent of blood is contained in blood agar?
5. Can agar slants be melted and reslanted? If so what would be the purpose of doing this?
6. At what temperature does agar melt?
7. At what temperature does agar solidify?
8. Define the following terms:
   - a. pH.
   - b. Sterilization.
   - c. Agar.
   - d. Broth.
   - e. Gelatin.

D. Preparation of Smears: Sensitivity Studies:

1. Preparation of smears:
   - a. Technique of preparing:
     - (1) Fixed smears.
     - (2) Hanging drop.
     - (3) Wet mounts.
   - b. Errors to be avoided.
   - c. Technique of preparing smears from:
     - (1) Sputum.
     - (2) Body cavity fluids.
     - (3) Exudates.
   - d. Preparation of loops and streaking equipment.
2. Sensitivity studies:
   - a. Preparation of plates for the addition of antibiotic discs.
   - b. Type of media to be used.
   - c. Application of material to plate.
   - d. Description of disc method.
   - e. Methods of dispensing discs:
     - (1) Single application.
     - (2) Machine dispenser.
   - f. Antibiotics used to test the sensitivity of:
     - (1) Gram negative organisms.
     - (2) Gram positive organisms.
     - (3) Staphylococcus.
     - (4) Others.
   - g. Reading results of disc sensitivity test.

Suggested reading assignments for students

Air Force Manual, Sec. 3, p. 6.
Bailey and Scott, pp. 26–27; Chapter 34.
Hepler, pp. 163–164.
Kimler, Chapter 34.
Levinson and MacFate, pp. 851–852.
Schaub and Foley, pp. 26–27.
Stewart, pp. 191–195.

Audiovisual Aids

Antibiotics, color, sound, 15 min., Encyclopaedia Britannica Films, Wilmette, Ill.

Bacterial Antibiotic Susceptibility Testing, color, sound, 25 min., Audio-Visual Library, ASMT Educational & Research Fund, Inc., Houston, Tex., 77025.

Laboratory Exercises

1. Practice making smears according to the procedure outlined in lecture.
2. Practice making smears from the following specimens according to the procedure used in your laboratory:
   - a. Sputum.
   - b. Broth.
   - c. Spinal fluid.
   - d. Urine.
   - e. Exudate.
3. Practice making wire loops and streaking equipment.
4. Outline in your notebook the correct procedure for making smears from the various laboratory specimens examined in your laboratory. Include a list of the errors to be avoided.
5. Draw and label the different kinds of loops and streaking apparatus used in your laboratory. Indicate when each would be used.
6. List the types of media used in your laboratory for antibiotic sensitivity studies.
7. Make a list of the antibiotic used in your laboratory to test the sensitivity of the following groups of organisms:
   - a. Gram positive.
   - b. Gram negative.
   - c. Staphylococcus.
   - d. Mixed flora.
8. Outline the procedure used in your laboratory for the determination of antibiotic sensitivity.
9. Outline the technique used in your laboratory for preparing a wet mount or hanging drop preparation.

Study Questions

1. Why must smears be fixed before applying stain?
2. What errors should be avoided in preparing smears?
3. What technique is used in dispensing antibiotic sensitivity discs to an agar plate by:
   a. Single disc application.
   b. Machine dispenser.
4. What type of media may be used for the determination of antibiotic sensitivity?
5. How should material to be exposed to antibiotics be applied to the agar plate?
6. What technique would you use for preparing smears from the following material:
   a. Sputum.
   b. Exudate.
7. For what purpose would a hanging drop preparation be made? How would you prepare such a slide?
8. For what purpose are fixed smears made?
9. How should zones of sensitivity and resistance be read?

E. Preparation of Stains and Staining Technique:

1. Principles and purposes of stains.
2. Gram strain—preparation:
   a. Gentian violet.
   b. Gram's iodine.
   c. Acetone-alcohol.
   d. Safranin.
   e. Other methods or modifications.
3. Gram staining techniques:
   a. Principle.
   b. Purpose of reagents.
   c. Technique.
4. Acid-fast stain (Ziehl-Neelsen)—preparation:
   a. Carbol fuchsin.
   b. Acid alcohol.
   c. Loeffler's methylene blue.
   d. Other methods or modifications.
5. Acid-fast staining technique:
   a. Principle.
   b. Purpose of reagents.
   c. Technique.
6. Capsule outline and capsule stain—preparation:
   a. India ink.
   b. Hiss method.
   c. Other methods or modifications.
7. Capsule staining technique:
   a. Principle.
   b. Purpose of reagents.
   c. Technique.
8. Flagella stain (Leifson Method Modified)—preparation:
   a. Basic fuchsin-tannic acid staining solution.
   b. Borax-methylene blue counterstain.
   c. Other methods or modifications.
9. Flagella staining technique:
   a. Principle.
   b. Purpose of reagents.
   c. Technique.
10. Spore stain (Wirtz-Conklin)—preparation:
   a. Malachite green.
   b. Safranin counterstain.
   c. Other methods or modifications.
11. Spore staining technique:
   a. Principle.
   b. Purpose of reagents.
   c. Technique.
12. C. diphtheriae stain—preparation:
   a. Neisser's stain.
   b. Albert's stain.
   c. Other methods or modifications.
13. C. diphtheriae staining technique:
   a. Principle.
   b. Purpose of reagents.
   c. Other methods or modifications.

Suggested reading assignments for students

Air Force Manual, pp. 3–2; 3–6.
Bailey and Scott, pp. 32–36.
Levinson and MacFate, pp. 944–949.
Schaub and Foley, pp. 491–501.
Simmons and Gentzkow, pp. 619–623.
Stewart, pp. 25–34.

Laboratory Exercise

Outline in detail in your notebook the technique used in your laboratory for doing the following:

1. Gram stain.
2. Acid-fast stain.

3. Capsule stain.
4. C. diphtheriae stain.

Study Questions

1. For what purpose would the following stains be used?
   a. Hiss stain.
   b. Ziehl-Neelsen stain.
   c. Gram stain.
   d. Albert's stain.
2. What reagents are used in the Gram stain? In what order are they used?
3. What reagents are used in the Ziehl-Neelsen stain? In what order are they used?
4. What is a mordant? Name some reagents that can be used as mordants.
5. Name some stains that can be used as counterstains.

F. Receiving, Handling, Processing, and Disposal of Infectious Material:

1. The receiving and handling of infectious material:
   a. Handling containers contaminated on outside.
   b. Types of disinfectants used in the bacteriology laboratory.
   c. Necessity and technique of properly sterilizing platinum loops and needles.
   d. Treatment of pipettes and capillary pipettes after exposure to contaminated material.
   e. Handwashing technique.
2. The processing and disposal of infectious materials:
   a. Protection of cultures and contamination.
   b. Labeling, coding, and entering of specimens in daily log book.
   c. Disposal of slides after smears have been read.
   d. Disposal of cultures after organisms have been isolated.
   e. Disposal of containers.
   f. Disinfection of a contaminated area.

Suggested reading assignments for students

Bailey and Scott, Chapter 7.
Hepler, p. 163.

Laboratory Exercises

1. Make a list of the types of disinfectants used in your laboratory.
2. Outline the procedure followed in your laboratory for disinfecting a contaminated area.
3. Outline the procedure used in your laboratory for receiving, handling, processing, and disposing of infectious material.

Study Questions

1. What part of the bunsen burner flame should be used for sterilizing inoculating needles and loops?
2. Name three types of disinfectants.
3. How would you protect cultures from contamination?
4. How would you go about disinfecting a contaminated area?
5. How should containers be handled if they are contaminated on the outside?

G. Preparation of Bacteriological and Serological Specimens for Shipment:

1. Shipment of bacteriological specimens:
   a. Types of specimens that may be shipped.
   b. Preparation of specimens:
      (1) Slants.
      (2) Filter paper method.
   c. Use of preservatives.
   d. Packing.
   e. Choice of container.
   f. Outside markings.
   g. Postal regulations.
2. Shipment of specimens in frozen state:
   a. Spinal fluid.
   b. Fecal specimens.
   c. Throat swabs.
   d. Tissue.
3. Preparation of serological specimens for shipment:
   a. Separation of serum using sterile technique.
   b. Preparation of serum.
   c. Sealing, labeling, wrapping, and packing of specimen.

Suggested reading assignments for students

Air Force Manual, Sec. 2, p. 13.
Bailey and Scott, Chapter 6.

Laboratory Exercises

1. Outline in detail how bacteriological specimens are prepared for shipment in your laboratory.
2. Outline the procedure used in your laboratory for the preparation and shipment of specimens in the frozen state.
3. Outline the procedure used in your laboratory for the preparation and shipment of serological specimens.

Study Questions

1. What kind of specimen would be sent to a reference laboratory in the frozen state?
2. What kinds of studies would be done on specimens preserved in the frozen state?
3. What technique would you use for separating serum from clotted blood?
4. Why is whole blood or serum not frozen for shipment?
5. What kind of preservative may be added to a fecal sample?

Unit III Serology

Suggested time: (See Bacteriology.)

Unit content

A. Technique of Withdrawing Blood.
B. Preparation of Tubes with and without Anticoagulant.
C. Preparation for and Performance of the VDRL Test.

Bibliography

Levinson, S. A., and MacFate, R. P., *Clinical Laboratory Diagnosis*, 6th ed., Philadelphia, Lea and Febiger, 1961.

Simmons, J. S., and Gentzkow, C. J., *Medical and Public Health Laboratory Methods*, Philadelphia, Lea and Febiger, 1956.

U.S. Dept. of Health, Education, and Welfare, Public Health Service, *Serologic Tests for Syphilis*, Washington, D.C., U.S. Government Printing Office (Publication #411), 1964.

Unit outline

A. Technique of Withdrawing Blood:

1. Syringes:
   a. Disposable.
   b. Multi-fit plunger.
   c. Single-fit plunger.
2. Needles:
   a. Disposable.
   b. Re-usable.
3. Vacutainer method:
   a. Types and sizes of tubes.
   b. Equipment.
   c. Preparation of equipment.
4. Technique of venipuncture:
   a. Preparation of patient.
   b. Preparation of needle and syringe.
   c. Application of tourniquet.
   d. Selection of vein.
   e. Application of antiseptic.
   f. Insertion of needle.
   g. Withdrawing the blood.
   h. Release of tourniquet and withdrawing the needle.
   i. Application of pressure to prevent bleeding.
5. Patient approach in:
   a. Isolation.
   b. Clinic and out-patients.
   c. Operating room.
   d. Delivery room.

Suggested reading assignments for students

Levinson and MacFate, pp. 242–243 and 559–561.

Simmons and Gentzkow, pp. 126–128.

Audiovisual Aids

(See appropriate films listed in Orientation Unit.)

Laboratory Exercises

To become proficient in the performance of a good venipuncture it is suggested that the students do the following:

1. At first, make early morning rounds with a technologist or I.V. nurse and observe:
   a. Approach to patients.
   b. Venipuncture technique.
   c. Tubes used for the collection of blood specimens.
2. Practice performing a venipuncture on one another using the steps outlined in lecture.
3. Practice performing a venipuncture on

new admissions and then clinic patients.

4. When sufficient skill has been obtained, perform venipunctures on hospitalized patients, at first under the supervision of a technologist or I.V. nurse and finally on their own.

Study Questions

1. What antiseptic is used for cleansing the site of a venipuncture?
2. What approach and technique would be used in obtaining blood from a patient in isolation?
3. Outline in detail the technique for performing a venipuncture.
4. What errors should be avoided in performing a venipuncture?
5. In the vacutainer method what piece of equipment represents the barrel of a syringe? What represents the plunger?
6. What precautions should be taken to avoid hemolyzing blood?

B. Preparation of Tubes with and without Anticoagulants:

1. Preparation of bottles and tubes containing anticoagulants:
   - a. Heparin.
   - b. Ammonium oxalate.
   - c. Potassium oxalate.
   - d. Sodium oxalate.
   - e. Sodium fluoride.
   - f. E.D.T.A.
   - g. Combinations of above.
2. Types of examinations done on whole blood:
   - a. Amount of blood to be added to tubes.
   - b. Hematological procedures (ESR, hematocrit, CBC, etc.).
   - c. Chemistry procedures (protein-free filtrate, etc.).
3. Types of examinations done on plasma:
   - a. How plasma is obtained.
   - b. Centrifugation and separation.
   - c. Hematology and chemistry procedures that are done on plasma.
4. Preparation of tubes without anticoagulant:
   - a. Size of tubes.
   - b. Addition of mineral oil.
   - c. Sterile tubes.
5. Types of examinations done on untreated blood:
   - a. How serum is obtained.
   - b. Centrifugation and composition.
   - c. Serological procedures done on serum.
   - d. Chemistry procedures done on serum.
   - e. Hematological procedures done on serum.

Suggested reading assignments for students

Levinson and MacFate, pp. 243-247.
Simmons and Gentzkow, p. 126.

Laboratory Exercises

1. Draw in your notebook a picture of the different types of bottles and tubes used in your laboratory for the collection of blood. Indicate the type of anticoagulant that is used for each.
2. Make a list of the type of procedures that can be done on the following:
   - a. Plasma.
   - b. Serum.
   - c. Whole blood.
3. If vacutainers are used in your laboratory, make a list of the different types of tubes used. Color code them, indicate the purpose for which they are used as well as the anticoagulants, if any, that have been added.

Study Questions

1. What is the difference between plasma and serum?
2. What type of blood specimens (whole blood, plasma, or serum) would be used for the following:
   - a. Blood sugar.
   - b. Protein.
   - c. Heterophil antibody.
   - d. Calcium.
   - e. Hematocrit.
   - f. Cholesterol.
   - g. Sedimentation rate.
   - h. Blood urea nitrogen.
   - i. Prothrombin determination.
   - j. Carbon dioxide determination.
3. Why is mineral oil sometimes added to

test tubes in which blood is to be collected?

4. What type of procedures require the use of a sterile tube?
5. What type of anticoagulant is considered the best for the majority of:
   - a. Hematological procedures.
   - b. Chemistry procedures.

C. Preparation for and Performance of the VDRL Test:

1. The handling and storage of specimens:
   - a. Receipt of specimens.
   - b. Numbering or coding of specimens.
   - c. Labeling of transfer tubes in racks.
   - d. Types of racks.
2. Separation of serum from clot:
   - a. Rimming the tube.
   - b. Technique of centrifugation.
   - c. Speed of centrifugation.
   - d. Transfer of serum to transfer tube.
3. Storage of specimen:
   - a. Plugging of tubes before storage.
   - b. Refrigeration (temperature).
   - c. Length of refrigerated storage time.
   - d. Deterioration of serum at room and refrigerated temperatures.
4. Introductory information regarding the performance of serological procedures:
   - a. Necessity of adhering to procedural technique.
   - b. Checking equipment:
     - (1) Water bath.
     - (2) Shaking and rotating machines.
     - (3) Centrifuges.
     - (4) Automatic pipetting machines.
   - c. Technique of cleaning glassware:
     - (1) Tubes.
     - (2) Slides.
     - (3) Pipettes.
   - d. Preparation of reagents:
     - (1) Antigens.
     - (2) Saline solution.
     - (3) Distilled water.
   - e. Temperature control:
     - (1) Room temperature.
     - (2) Refrigerated temperature.
   - f. Terminology used in reporting results:
     - (1) Reactive.
     - (2) Weakly reactive.
     - (3) Nonreactive.
   - g. Preparation of control serum:
     - (1) Reactive.
     - (2) Weakly reactive.
     - (3) Nonreactive.
5. Preparation for the performance of the VDRL test:
   - a. Equipment:
     - (1) Rotating machine.
     - (2) Hypodermic needle of correct size with and without point.
   - b. Glassware:
     - (1) Slides, 2x3 inches.
     - (2) Antigen emulsion bottles.
     - (3) 1-2ml syringe.
   - c. Reagents (antigen and saline solutions):
     - (1) Preparation and storage of antigen emulsion.
     - (2) Testing antigen emulsion.
     - (3) Delivery needles.
     - (4) Preliminary testing of antigen emulsion.
     - (5) Preparation of saline solution.
   - d. Preparation of sera:
     - (1) Separation from clot.
     - (2) Centrifugation.
     - (3) Inactivation.
6. VDRL slide qualitative test:
   - a. Technique of test.
   - b. Serum controls.
   - c. Reading and reporting test results.
   - d. Errors to be avoided.

Suggested reading assignments for students

Serologic Tests for Syphilis, pp. 1–7; 119–125.

Audiovisual Aids

VDRL Test for Syphilis, B & W, sound, 23 min., Audiovisual Div., Communicable Disease Center, Atlanta, Ga.

Laboratory Exercises

1. Outline in your notebook the procedure used in your laboratory for checking the following:
   - a. Rotation of shaking machine.
   - b. Speed of centrifuge.
   - c. Temperature of water bath.
   - d. Antigen emulsion delivery needle.
2. Outline in your notebook the procedure

used in your laboratory for cleaning the following:
  - a. Pipettes.
  - b. Slides.
  - c. Bottles.
3. Outline in your notebook the procedure used in your laboratory for preparing the following:
  - a. Antigen emulsion.
  - b. Control serum.
  - c. Sera to be tested.
4. Outline in your notebook the procedure for doing a qualitative slide VDRL test.

Study Questions

1. What is the size of the syringe used in the VDRL test?
2. What gauge and length needle is used? Does it have a point?
3. How many oscillations should the shaker make per minute?
4. What kind of shaker is used?
5. What terminology is used in reporting the results of the VDRL test?
6. How long may antigen remain at room temperature before it is no longer usable?
7. How should slides be cleaned prior to use for the VDRL test?
8. How should the antigen emulsion be prepared?
9. What is the pH of the saline that is used?
10. What is the purpose of inactivating sera before testing?
11. At what temperature and length of time are sera inactivated?
12. How long may sera remain at room temperature before they must be reheated?

Unit IV Parasitology

Suggested time: (See Bacteriology.)

Unit content

A. Collection, Handling, and Preparation of Fecal Specimens.

B. Performance of a Concentration Test.

Bibliography

Belding, D. L., *Textbook of Clinical Parasitology,* New York, Appleton-Century-Crofts, 1952.

Faust, C. C. and Russell, P. F., *Craig and Faust's Clinical Parasitology,* Philadelphia, Lea and Febiger, 1964.

Levinson, S. A., and MacFate, R. P., *Clinical Laboratory Diagnosis,* 6th ed., Philadelphia, Lea and Febiger, 1961.

Simmons, J. S., and Gentzkow, C. J., *Medical and Public Health Laboratory Methods,* Philadelphia, Lea and Febiger, 1956.

Manuals

Department of the Army, *Laboratory Procedures in Parasitology,* Technology Manual, TM 8–227–2, Washington, D.C., U.S. Government Printing Office, 1961.

Catholic Hospital Association, Committee on Medical Technology, *Parasitology, A Diagnostic Workshop for Medical Technologists,* The Association, St. Louis, Mo.

Unit outline

A. Collection, Handling, and Preparation of Fecal Specimens:
1. Collection or receipt of fecal specimens:
  - a. Warm stool:
    - (1) Purgation.
    - (2) Proctoscopic material.
  - b. Preserved stool:
    - (1) Polyvinyl alcohol fixative (PVA).
    - (2) Merthiolate-iodine-formalin (MIF).
  - c. Pinworm:
    - (1) N.I.H. swab.
    - (2) Scotch tape mount.
2. Handling of fecal specimens:
  - a. Macroscopic examination for:
    - (1) Blood.
    - (2) Mucous.
    - (3) Adult worms.
    - (4) Barium or oil.
3. Preparation of fecal specimens:
  - a. Wet preparation.
    - (1) Saline.
    - (2) Iodine.
    - (3) Errors in preparation.

Suggested reading assignments for students

Levinson and MacFate, pp. 119–123.

Simmons and Gentzkow, pp. 888–889.

Laboratory Exercises

Outline in your notebook the procedure used in your laboratory for preparing the following:

1. Saline slide.
2. Iodine slide.
3. Scotch tape mount.

Study Questions

1. What errors should be avoided in the preparation of wet smears?
2. What type of worms might be found on gross examination of a stool specimen?
3. From what sources is material for parasitological examination obtained?
4. For what purpose is a scotch tape mount or N.I.H. swab used?

B. Performance of a Concentration Test:

1. Formalin-Ether sedimentation (Ritchie Technique):
    - a. Equipment:
        - (1) Centrifuge.
        - (2) 15ml centrifuge tubes.
        - (3) Gauze.
    - b. Reagents:
        - (1) Saline.
        - (2) 10-percent formalin.
        - (3) Ether.
    - c. Preparation of stool:
        - (1) Emulsification.
        - (2) Straining.
        - (3) Centrifugation.
    - d. Technique of test:
        - (1) Addition of reagents.
        - (2) Centrifugation.
        - (3) Errors to be avoided.

Suggested reading assignments for students

Simmons and Gentzkow, pp. 887–888.

Laboratory Exercises

1. Practice the techniques described in lecture.
2. Outline in your notebook the procedure used in your laboratory for doing a concentration test for parasitological examination.

Study Questions

1. How many layers result after the addition of ether and centrifugation?
2. What percent formalin is used in the formalin-ether concentration method?
3. What errors should be avoided when doing this test?
4. For what reason is a concentration test employed?
5. How much fecal sediment should be present after initial centrifugation?

Unit V Hematology

Suggested time: 10 weeks; 400 hours.

Introduction

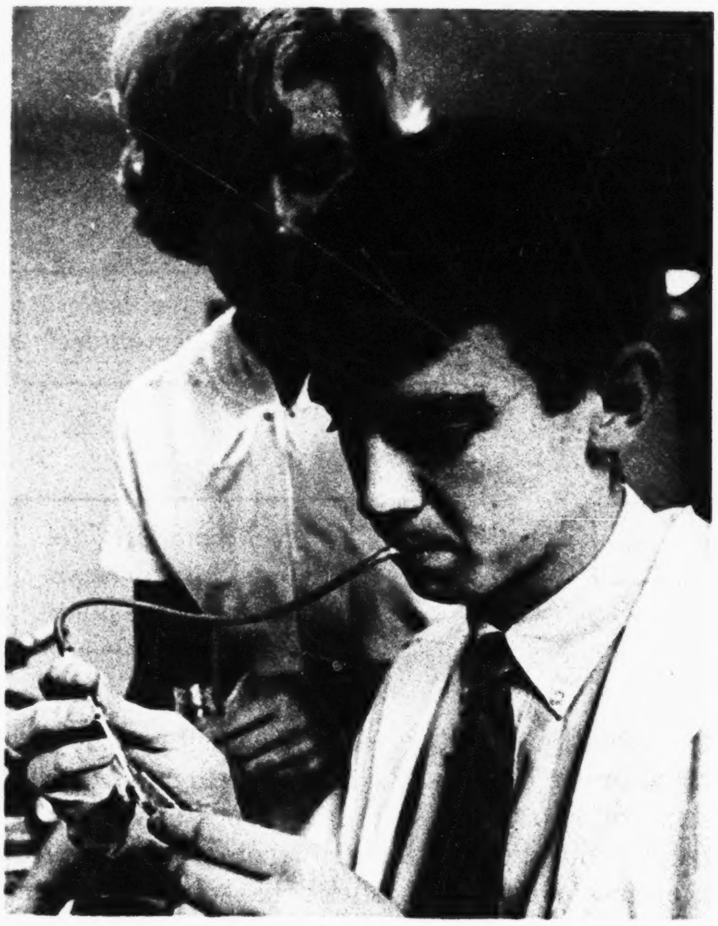
The material to be presented to the student has been outlined under 11 headings. It is believed that a minimum of 20 class periods should be used to cover this basic background. Each section has accompanying practice sessions to allow the student to learn the techniques related to the theory which he studies. In outlining the year's course, a maximum number of hours must be stated for each practice session, but the instructor will find that some students should have additional practice time in order to become proficient in the techniques. The outlined content for each section gives the instructor guidelines to follow in materials and subject matter found in most books of laboratory techniques.

Unit content

A. Introduction to Hematology.
B. The Blood Count.
C. Hemoglobin.
D. Methods for Collection of Blood.
E. Blood Smears.
F. General Techniques and Discussion of Blood Smears.
G. Origin and Relationship of Blood Cells.
H. Leukocytosis and Leukopenia.
I. Coagulation.
J. Hematocrit, Cell Indices, and Sedimentation Rate.
K. Anemias.

Bibliography

Bordewich, Patricia H., "Quality Control in Methods in a Routine Hematology Laboratory," *Postgraduate Medicine*, 33:5, May 1963. (Also helpful for instructors is a paper by Patricia H. Bordewich, "Statistical Evaluation of Student Technical Performance,"



mimeographed by and available from Hematology Laboratory, University of Minnesota Hospital, Minneapolis, Minn.)

Bray, W. E., *Clinical Laboratory Methods*, 6th ed., St. Louis, C. V. Mosby Co., 1962. (This book is well-organized and should not be too difficult for student assignments. However, a few errors, such as in calculation of Hemoglobin standards on page 128, have been noted. The instructor should study assignments carefully so as to call such errors to the attention of the student.)

Davidsohn, I., and Wells, B. B., *Clinical Diagnosis by Laboratory Methods*, 13th ed., Philadelphia, W. B. Saunders Co., 1962.

Manual for Teaching Blood Coagulation Techniques in the Routine Laboratory, General Diagnostic Div., Warner-Chilcott Co., Morris Plains, N.J.

Page, L. B., and Culver, P. J., *A Syllabus of Laboratory Examinations in Clinical Diagnosis*, Cambridge, Harvard University Press, 1961. (This is a preferred text for the students. It may seem too difficult for the laboratory assistant student at first, but with adequate classroom instruction and concentration on the student's part, the quality of information gained outweighs the difficulty encountered.)

Watson, C. J., ed., *Outlines of Internal Medicine*, 9th or 10th ed., Parts IV and V, Dubuque, Iowa, Wm. C. Brown Co., 1958 or 1963.

Wells, Benj. B., *Clinical Pathology*, 3d ed., Philadelphia, W. B. Saunders Co., 1962.

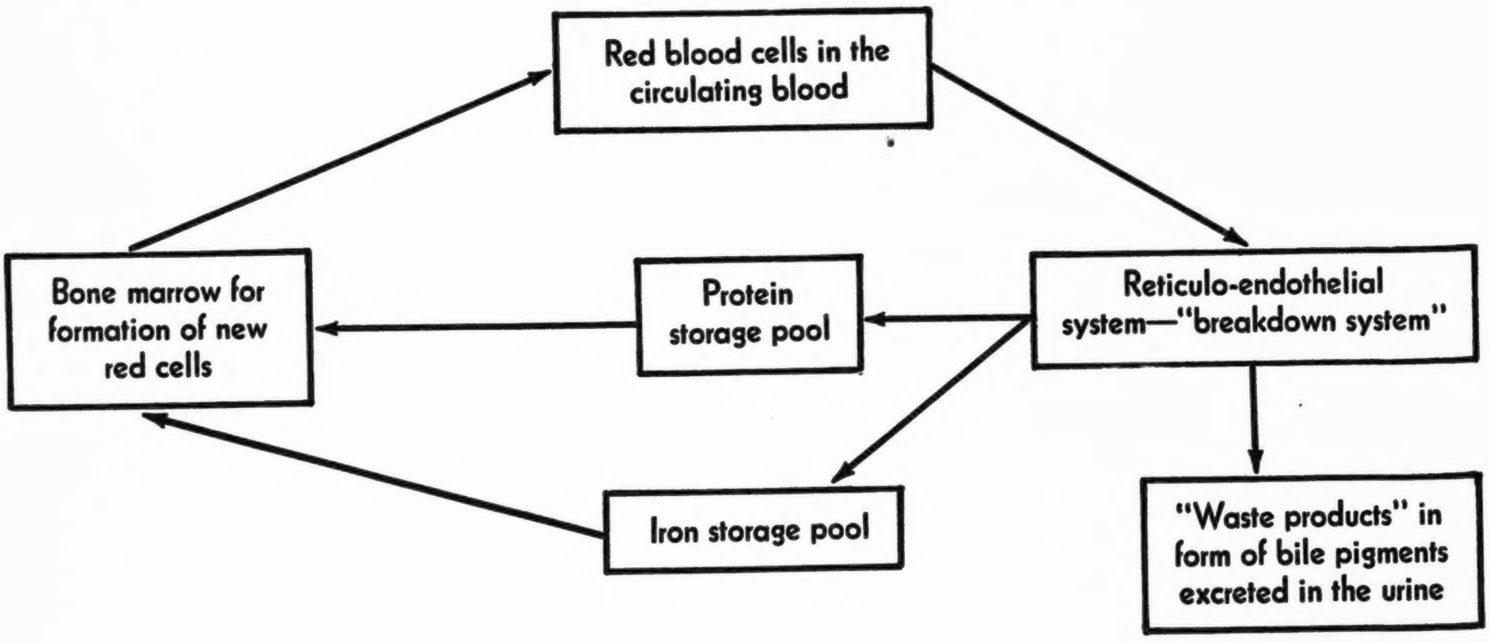
Wintrobe, Maxwell M., *Clinical Hematology*, 5th ed., Philadelphia, Lea and Febiger, 1961.

Unit outline

A. Introduction to Hematology:
  1. Formation and destruction of blood:
    a. Detailed discussion of the following schematic representation:
    b. The three major cell types of the blood—simple description and explanation of:
      (1) Erythrocyte—red cell.
      (2) Leukocyte—white cell.
      (3) Thrombocyte—platelet.
  2. Blood anticoagulants:
    a. The function and use of anticoagulants.
    b. The kinds of anticoagulants and how to use each.
  3. Blood cell diluents:
    a. Principles of hemolysis, with discussion of osmosis and isotonic, hypertonic, hypotonic, and physiologic solutions.
    b. Qualities and use of diluents:
      (1) RBC diluents with discussion of Hayem's solution, 0.85 percent saline, Gower's solution, Toison's solution, and Rees-Ecker solution.
      (2) WBC diluents with discussion of 2 percent acetic acid and 0.1 N hydrochloric acid.
    c. Red cell count.
  4. Blood cell pipettes:
    a. General qualifications of manufacture.
    b. Units of measure of a red cell and a white cell.
    c. Calculation of dilution factors in the use of pipettes.

Audiovisual aids and demonstration material

1. Diagrams of the pipettes presented on lantern slides or drawn on the blackboard by the instructor.


Red blood cells in the circulating blood
Reticulo-endothelial system—"breakdown system"
Protein storage pool
Bone marrow for formation of new red cells
Iron storage pool
"Waste products" in form of bile pigments excreted in the urine

2. Pictures of the three cell types.
3. Tubes (or a lantern slide of tubes) showing layers of blood elements.
4. Tubes containing anticoagulant with the identifying markings used in your hospital.
5. One tube of blood with anticoagulant and one without, to illustrate the difference in appearance.

Laboratory Exercises

The objective of laboratory exercises accompanying this lecture unit should include recognition by the student of the importance of proper care in handling blood specimens, using the prescribed method for cleaning pipettes and understanding the common precautions and errors in pipetting. The student should learn to dilute both a red and a white pipette correctly and not be allowed to proceed until this is accomplished. Individual instruction is essential at this time.

Equipment Needed: At least 5 red and 5 white pipettes for each student, diluting fluid bottles and filtering equipment, diluting fluids, necessary material for cleaning pipettes, gauze or tissues, a sample of anticoagulated blood.

Exercises. The student should be given work sheets with directions to filter diluting fluids and practice diluting pipettes until he has completed 5 perfect pipettes of each type and had the technique and results checked by the instructor before results are recorded. The instructor should allow only perfect pipettes for credit and should feel upon completion of the exercises that the student has mastered the technique of diluting red and white cell pipettes.

Study Questions

1. What is the two-fold purpose of the white cell diluting fluid?
2. Name two fluids that can be used to dilute red cells, other than Hayems.
3. Why and how (decrease or increase) would a bubble in the pipette affect the count if the pipette were used?
4. What are the criteria for a good red cell diluent?
5. Why is the white cell pipette shaken vigorously after being diluted?
6. Give the proper name for the three formed elements of the blood.
7. Platelets are produced in the bone marrow by what cell?
8. Blood drawn by needle and syringe is kept from clotting by use of an ________________. Name two used for preserving hematology specimens. Which one is better, and why?
9. What are the waste products of red cell breakdown which are excreted in the urine and feces?

10. Which products of red cell breakdown are stored and re-used by the body?
11. Define osmosis.
12. When blood is mixed with dilute acetic acid, the mixture turns brown. Why?
13. Give the abbreviation and the full chemical name for sequestrene. (Found in reading in Page and Culver.)
14. Define osmotic pressure.
15. What happens to the red cells when the solution in which they are suspended is (a) hypotonic, (b) hypertonic?
16. Explain how to calculate dilution factors for the white blood count as it is done routinely.
17. What appearance of the plasma would indicate hemolysis of the red cells? Why does the plasma have this appearance?

B. The Blood Count:

1. Counting chamber:
   - a. Detailed explanation of all dimensions of the counting chamber.
   - b. Diagrammatic representation (see Page and Culver, pp. 28, 29, 30):
     - (1) Discussion of difference in Levy-Hausser chamber with Neubauer ruling, the Spencer Bright-Line, and the Fuchs-Rosenthal chambers.
     - (2) Proper cleaning of hemacytometer.
2. Performance of red and white cell counts:
   - a. Use of the hemacytometer:
     - (1) Necessity for proper shaking and the proper method to use in mounting counts is generally discussed, with more emphasis being placed on these functions during laboratory exercises.
   - b. White cell count:
     - (1) Routine method of counting cells, with diagrammatic illustration.
     - (2) Calculation of the count, using dilution factors.
     - (3) Normal values.
     - (4) Sources of error.
   - c. Red cell count:
     - (1) Routine method of counting cells, with diagrammatic illustration.
     - (2) Calculation of the count, using dilution factors.
     - (3) Normal values.
     - (4) Sources of error.

Laboratory Exercises

The objectives of this section should include a review of the proper mixing and pipetting of blood, the proper care of the counting chamber, the proper use of the microscope with the counting chamber, developing and practicing the proper routine for mounting and counting, and understanding the calculations for a count. The student should learn to meet exact standards in performance of counts. The following criteria are suggested: for white cell counts, the allowable difference between duplicate pipettes on a single sample should be 500 cells per cu. mm for counts within normal range, and 10 percent of the lowest count when the total is less than 500 or over 10,000. Duplicate red cell counts must agree within 10 percent.

Equipment Needed: Pipettes from the previous exercise, diluting fluids, samples of blood with known counts, counting chambers and cover glasses, and microscopes.

Exercises: The student should be required to practice counting until the instructor has observed technique and is satisfied with results to the extent that each individual student is considered to possess adequate skill to allow him to do patient work on preserved blood under strict supervision in the laboratory.

Study Questions

1. What kind of count would you obtain if the first three drops were not expelled from the pipette before mounting?
2. What happens to the white cells in the blood when you do a dilution for a red blood cell count?
3. List three types of counting chambers.
4. What is meant by the term "cell distribution"?
5. Why can you not do the following things: (a) Remove the excess liquid if you have too much on the counting chamber after the pipette has been mounted? (b) Add another drop if the first one did not completely fill the ruled area? (c) Keep using the same pipette repeatedly if the mount was not satisfactory the first two times?

(d) Adjust back to the 11.0 or 101.0 mark if you over-dilute the pipette?

6. Calculate the dilution factors for the following (show work): (a) Blood drawn to the 0.2 mark and diluted to the proper mark in a red blood pipette. ____________ (b) Blood drawn to the 1.0 mark and diluted in the usual manner in a red pipette. ____________ (c) Blood drawn to the 0.1 mark and diluted in a white cell pipette. ________ (d) Blood drawn to the 0.4 mark and diluted in a red cell pipette. ______

C. Hemoglobin:

1. Hemoglobin:
   a. Simplified discussion of structure.
   b. Types of hemoglobin. Brief discussion of oxyhemoglobin, bethemoglobin and carboxyhemoglobin.
   c. Some terms related to hemoglobin and conditions associated with its alteration:
      (1) Anemia.
      (2) Erythrocytosis.
2. Determination of hemoglobin:
   a. Visual methods:
      (1) Discussion, mostly from historical standpoint, of Sahli, Dare, and Tallquist methods.
   b. Calorimetric methods:
      (1) Oxyhemoglobin—technique and discussion.
      (2) Cyanmethemoglobin—technique and discussion.
      (3) Van Slyke method discussed briefly.
   c. Reporting of hemoglobin and interpretation of values:
      (1) Discussion of reporting hemoglobin in gm/100 ml vs. percent.
      (2) Correlation of hemoglobin and red count.

Visual aids and demonstration material

1. Demonstration of visual hemoglobin method.
2. Lantern slides to show reason for correlation of red count and hemoglobin to be discussed only briefly.
3. The same type of material could be used in discussing anemia.
4. Review of operation of colorimeter.

Laboratory Exercises

The objective of these laboratory exercises is mainly to introduce the student to the techniques of doing hemoglobin concentration after he has been doing cell counts and has a good working knowledge of the use of the colorimeter. Stress is placed on technique; standardization procedures, preparation of control solutions, and understanding correlation of red cells and hemoglobin values are given near the end of the training period. The exact method used to instruct the student will depend upon which one is being used routinely in the institution. He should be allowed to perform at least one visual and one colorimetric method.

Equipment Needed: Several hemoglobin pipettes, diluting fluid, reading instruments, and samples of blood on which the hemoglobin value is known.

Exercises: The student should do several hemoglobin determinations until four replicates agree within 0.5 gm/100 ml. on each specimen, for each of two methods used in the exercise. Stress should also be placed on the comparative values themselves to point out the great variations possible.

Study Questions

1. List the methods in addition to oxyhemoglobin for the determination of hemoglobin concentration.
2. What are the disadvantages or advantages of these methods?
3. For what total amount is the Sahli pipette calibrated in cu mm? What would the dilution be if the blood measured in a Sahli pipette is placed in a total of 5 ml of diluent? In 10 ml of diluent?
4. What are the 100 percent and normal values for the oxyhemoglobin method?
5. An oxyhemoglobin determination was found to be 14.5 gm/100 ml. Is this value decreased, increased, or normal?
6. Is the Sahli pipette calibrated to contain or to deliver?

D. Methods for Collection of Blood:

1. Collection of peripheral samples:

- a. Preparation of equipment.
- b. Preparation of patient.
- c. Technique of obtaining sample.
- d. Delivery to the laboratory.
- e. Storage in the laboratory.

2. Collection of venous samples:
   - a. Preparation of equipment.
   - b. Preparation of patient.
   - c. Technique of obtaining sample.
   - d. Delivery to the laboratory.
   - e. Storage in the laboratory.
   - f. Importance of prompt and proper handling of venous sample and effects on all hematologic determinations.

Audiovisual aids and demonstration materials

1. Lantern slides to illustrate the technique of finger-prick and venipuncture are good.
2. Observation of medical technologists collecting blood in the clinic and in the hospital.

Laboratory Exercises

Some instruction in finger puncture may have been combined with the training technique, but stress on collection should be reserved for this unit. The counting techniques give the student a purpose for blood collection.

Equipment Needed: Usual collecting equipment. The student should make complete preparation for use.

Exercises: The students must make complete preparations for doing finger pricks and venipunctures. They can practice on each other under careful observation and instruction of the teacher, and if possible on volunteers from other sources. When a student has made several punctures, he should be able to do venipunctures on selected patients under supervision. In doing finger pricks, several collections should be done and the counts checked for accuracy. The supervisor should take duplicate counts from the patient at the same time as the student, until these checks are within range.

Study Questions

The instructor should prepare questions adjusted to the manner in which the techniques are taught.

E. Blood Smears:

1. Purpose of the blood smear.
2. Preparation for the smear:
   - a. Equipment.
   - b. Collecting the blood.
   - c. Making the smear—slide and cover slip.
   - d. Criteria of a properly prepared smear.
3. Staining the smear:
   - a. The Wright's staining method, stressing the importance of each step, the pH of the buffer, and timing.
   - b. Recognition of a properly stained smear.
4. Examination of the stained smear:
   - a. The importance of examination of the gross appearance of smear.
   - b. Overall scanning of smear on low power:
     - (1) Proper distribution of all cells.
     - (2) Correlation of cell distribution with total white cell count.
   - c. Examination of smear under oil immersion lens:
     - (1) Red cells—size, shape, color, inclusions.
     - (2) Platelets—size, number.
     - (3) White cells—differentiation of age.
     - (4) Nucleated red cells—identification, correction of white count.
   - d. Correlation of study with other determined values, such as total white count, red count, hematocrit, and hemoglobin values. Emphasis is to be placed on correlation from a technical standpoint, to imply accurate and inaccurate (reasonable and unreasonable) as well as normal and abnormal.

Visual aids and demonstration materials

1. Lantern slides and microscopic slides are invaluable from the time this exercise has begun until completion of the entire section. One source which may be helpful is the Hematology Correspondence Courses available from the Department of Post-graduate Medical Education, University of Kansas School of Medicine, Kansas City 12, Kansas. For laboratory students, Part I may be more useful than Part II.

2. For this section, slides should be shown to illustrate RBC's which are normocytic, microcytic, macrocytic; also, poikilocytosis, anisocytosis, Howell-Jolly bodies, polychromasia, and basophilic stippling. The diseases are not discussed at this time.

3. Use miscellaneous smears showing the difference between well-prepared and stained smear as contrasted to poorly prepared and poorly stained smears.

Laboratory Exercises

The objective of this section is to make each student aware of the need for properly prepared and stained slides as well as to make him proficient in collection and staining. The erroneous impressions given by poorly prepared and/or poorly stained smears should be stressed.

Equipment Needed: Slide carriers for the student, uncleaned slides, Wright's staining solution and buffer, staining racks, microscopes, and immersion oil.

Exercises: This study should be divided into at least three sessions, with the first session spent entirely on preparation of slides and making of the smear, the second session on staining the smear and evaluating the stain, and the third session on evaluation of the smear. The sessions should include fingerprick slides as well as slides from preserved blood. CBC's must be done on the samples in order to correlate results. For each assigned specimen, the student should complete a work sheet and hand it in with the slide, so that the instructor can check his results and interpretations. A suggested work sheet follows:

1. Hemoglobin ________ gm/100 ml percent. WBC __________. RBC __________.
2. Observation of stained smear: Estimated white cell count __________. Does this agree with the actual total white count? __________. Morphology and estimated number of platelets ________. Morphology of red cells ________. List the types of white cells you saw on your slide. __________. Considering the hemoglobin value and the appearance of the red blood cells (size and hemoglobin content), what do you think the red cell count would be?

Study Questions

1. Definition: anisocytosis, poikilocytosis, hypochromasia, polychromasia, thrombocytosis, leukopenia.
2. Some review problems:
   a. On diluting a white cell pipette from the 0.4 mark to the 11.0 mark, you counted the 4 corner squares and the center square and found a total of 180 cells. This gives a total count of __________.
   b. Calculate the cell counts using the following information:
      (1) Cells 42, 52, 46, 48. Area—same as for routine WBC. Depth—0.1 mm. Dilution—same as for routine WBC. Result __________.
      (2) Cells—102, 104, 104, 110. Area—4 rows across in center square mm. Depth—0.1 mm. Dilution—same as for routine WBC. Result __________.
3. Name two situations in which a blood smear is done even if it is not requested specifically by the physician.
4. How many smears are taken with each blood count, and why?
5. Name three sources of blood for a blood smear.
6. What would you do if your blood smear was too thin?
7. Why would you find it hard to count a blood smear which is too thick?
8. What general term indicates a decrease of hemoglobin concentration below the normal value?

F. General Techniques and Discussion of Blood Smears:

1. Review of low-power appraisal of smear.
2. Morphological separation of anemias:
   a. Hypochromic anemias.
   b. Normochromic anemias.
   c. Macrocytic anemias.
3. Platelets:
   a. Increased—significance.
   b. Decreased—significance.
4. Leukocytes:
   a. Leukopenia.
   b. Leukocytosis.
   c. Classification of leukemias.

Audiovisual Aids

Slides, both lantern and microscopic, to illustrate various findings and changes in blood cells.

Laboratory Exercises

The objective of this section is to eliminate completely the textbook attitude of blood smear observation by the student. Differential counting should be postponed until the student realizes that the actual classification of white cells is only a part of utilizing a blood smear. The laboratory assistant has a very important and critical role to play in *screening patient slides.* He must understand that the initial screening often means the difference between adequate treatment for the patient or prolonging recovery by not having the necessary information available for the physician to use.

Equipment: Stained smears of all types.

Exercises: Continued practice on exercises previously described, requiring the student to correlate hemoglobin, white cell, platelet, and red cell with each of the other pertinent procedures and with Wright's stained blood smears.

Study Questions

The instructor can best prepare problem questions to fit study slides.

G. Origin and Relationship of Blood Cells:

1. Formation of the blood cells:
   a. Fetal blood formation and immediate post-birth.
   b. First years of life to maturity.
   c. Aspiration of bone marrow—purpose and technique.
2. Identification of stained blood cells:
   a. Classifications:
      (1) Origin.
      (2) Age:
         (a) Change in size.
         (b) Change in intracellular structure.
         (c) Change in staining properties.
         (d) Evidence of cell function.
   b. How to do a differential:
      (1) Number of cells to count.
      (2) Recording of cell types.
      (3) Calculation of count.

Laboratory Exercises

The objective of this section is to train the laboratory assistant student in the counting of a normal differential. Only the classification of young cells as "young" is to be stressed. In other words, *any* change from the normal should be turned over to someone with more training and experience.

Exercises: On a preserved blood sample, the student should do a hemoglobin determination and a red cell count in duplicate and make two smears, staining one of the smears and handing both in with his work sheet. A complete white cell differential is done on the stained smear and the student is to give a description of the appearance of the red blood cells, platelets, and general appearance of the slide. A work sheet is prepared to record all information.

The same procedure is carried out on a smear obtained from a finger prick done on a fellow student.

The results should agree with the results obtained by the instructor (who can do the red counts on an electronic counter). The finger prick results can be checked by observing the smear turned in by the student. Points should be taken away if the student does not complete each result with the proper unit such as cells/cu mm gm/100 ml, or percent.

Study Questions

1. Review questions on any procedure completed to date.
2. Questions in the area of differential counting:
   a. The largest blood cell of the normal peripheral blood is the ________.
   b. In a poorly made blood smear, the differential count results would probably show a falsely high percentage of which cell type?
   c. Poikilocytosis refers to ________________________.
   d. Smears stained with Wright's stain are chemically fixed with ________.
   e. When Wright's stain is too acid, the erythrocytes will stain ________.
   f. A reticulocyte is ________________.

H. Leukocytosis and Leukopenia:

1. Leukocytosis:
   a. Definitions — neutrophilic, absolute, relative leukocytosis, eosinophilia, lymphocytosis, monocytosis.
   b. Physiological leukocytosis.

- c. Neutrophilic leukocytosis—discussion of conditions in which this state occurs: acute infestions, intoxications, pregnancy, acute hemorrhage, post-surgery, malignant growth, and myelogenous leukemia.

2. The hemogram:
   - a. Arneth hemogram—its development, how it is used, meaning of "shift to left" and "shift to right."
   - b. Schilling hemogram—its development, how it is used, meaning of "shift to left" and "shift to right."
3. Infectious processes:
   - a. Character of responses to infection:
     - (1) Intensity of stimulus.
     - (2) Reacting power of individual.
   - b. Schilling concept:
     - (1) Neutrophilic battle phase.
     - (2) Monocytic defense state.
     - (3) Lymphocytic cure stage.
4. Significance of various blood changes occurring in infections:
   - a. Eosinophilia.
   - b. Basophilia.
   - c. Leukopenia.
   - d. Lymphocytosis.
   - e. Monocytosis.

Demonstration material

The student will be carrying out exercises listed below during this presentation, and discussions will point out findings on smears which coincide with lecture material presented.

Laboratory Exercises

The objective of this section is to have the student become proficient in recognizing a deviation from the normal in a blood smear.

Equipment Needed: Differentials and evaluation of same.

Exercises: During the time of presentation of the preceding information, the student can complete a project which consists of evaluating 25 differentials. Each student is assigned a box of prepared slides for which the differentials have been completed by the instructor. Each result is checked for similarity of findings and any gross difference is discussed with the student. A work sheet is designed for use and is turned in with the slides.

Study Questions

1. What general factors are taken into consideration in order to differentiate the various types of white cells on a stained blood film?
2. Name the leukocytes that are classified as granulocytes.
3. What are some other names for neutrophil?
4. What are the components of Wright's stain?
5. What should one do if a differential count shows abnormal percentages in the following situations:
   - a. The neutrophils are increased.
   - b. The count shows a marked lymphocytosis.
6. If there is any deviation on the blood smear from the "normal," either in percentage or morphology, what is the first responsibility of the laboratory assistant?
7. What are the general criteria for determining whether a blood cell is *young*?

I. Coagulation:

1. Introduction to coagulation: Simplified visual clotting not indicative of complex reactions involved; clotting mechanism only as effective as influencing factors, just as a building is only as strong as its foundation.
   - a. Responsibility of the laboratory to detect which of the factors is weak.
   - b. The more complicated tests—usually done by specially trained medical technologists; duties of the laboratory assistant involve more routine tests.
2. Factors influencing classification of hemorrhagic disorders:
   - a. Vascular factor:
     - (1) Contraction and retraction of injured vessel is first line of defense against hemorrhage. Muscle cells in all vessels except capillaries.
     - (2) Capillary permeability:
       - (a) Injury and consequent formation of petechiae.
       - (b) Relationship of Vitamin C,

steroid hormones; non-specific relation to spleen.

- b. Platelet factor—platelets contribute to hemostasis in following ways:
  - (1) alone, or with fibrin, form hemostatic plug.
  - (2) participate in blood coagulation process itself.
  - (3) may be essential to prevent passage of blood cells through capillary walls.
  - (4) may carry a local vasoconstrictor identified as serotonin.
- c. Formation of clot.
  - (1) First two factors stop or slow flow of blood, clot forms and stops flow and prevents renewed bleeding when vascular factor relaxes.
  - (2) Brief discussion of three stages of fibrin formation and factors involved—AHF, PTC, PTA, L.F., S.F.
  - (3) Role of calcium.
  - (4) Natural coagulation elements in circulating blood.
  - (5) Coagulation elements present in tissues.
- d. The removal of fibrin from the blood.

3. Tests to detect deficiencies in coagulation factors:
   - a. Tests for vascular factor:
     - (1) Cuff test (tourniquet test, capillary resistance test, Rumpel-Leede) with principle, indications, technique, and limits of interpretation.
     - (2) Bleeding time with principle and explanation of tests:
       - (a) Duke method.
       - (b) Ivy method.
       - (c) Limitations and interpretations of bleeding tests.
   - b. Tests for platelet factor:
     - (1) Platelet counts—interpretation and limitations.
     - (2) Bleeding times—interpretations.
     - (3) Clot retraction—interpretation.
     - (4) Prothrombin consumption test—describe later.
   - c. Tests for coagulation mechanism:
     - (1) Coagulation time of venous blood —limitations: (Lee-White method, technique and interpretation.)
     - (2) Screening test to point out plasma factor deficiency or an anticoagulant present—interpretation.
     - (3) Prothrombin time—interpretation.
     - (4) Prothrombin consumption test—interpretation.
     - (5) Thromboplastin generation test—defined.
     - (6) Increased fibrinolysis—interpretation.
     - (7) Fibrinogen concentration—defined.

Suggested reference for student assignments

Page and Culver, p. 193, study Table 38; p. 194, review tests in Table 39.

Laboratory Exercises

Class work is limited in this section since actual practice comes in department service. The students should observe demonstrations of bleeding times, clotting times, and cuff tests. It should be the instructor's decision whether actual practice should be done in these tests.

Study Questions

1. How do the anticoagulants we use in hematology work to preserve blood specimens and to prevent coagulation of blood?
2. State the physiologic function tested in the following tests:
   (a) Tourniquet test; (b) Bleeding time; (c) Whole blood clotting; (d) Prothrombin time.
3. A peripheral blood smear which you examined appeared normal but for the marked decrease in platelets. When this observation was reported and subsequently confirmed by a platelet count, patient's physician requested the following procedures: a. Tourniquet test; b. Whole blood clotting time; c. Prothrombin time. What results would you anticipate in each instance?
4. Dicumarol and heparin (therapy) both act on the blood clotting mechanism by ______________.
5. Which test in the evaluation of coagula-

tion is used to follow a patient receiving dicumarol therapy, and why?

6. A Vitamin K deficiency would be likely to cause ________________.

J. Hematocrit, Cell Indices, and Sedimentation Rate:

1. Hematocrit:
   a. Definition and use.
   b. Methods:
      (1) Micro method—technique and sources of error.
      (2) Macro method—technique and sources of error.
      (3) Normal values and interpretations.
2. Red cell indices:
   a. Mean corpuscular volume (MCV)—use, calculation, interpretation.
   b. Mean corpuscular hemoglobin (MCH)—use, calculation, interpretation.
   c. Mean corpuscular hemoglobin concentration (MCHC)—use, calculation, interpretation.
   d. Color index—use, calculation, interpretation.
3. Erythrocyte sedimentation rate (ESR):
   a. Principles of the test:
      (1) Physiological factors.
      (2) Curve of fall.
      (3) Effect of anemia on curve.
   b. Use of the sedimentation rate:
      (1) Increase in rate—meaning.
      (2) Decrease in rate—meaning.
   c. Methods:
      (1) Wintrobe and Westergren.
      (2) Sources of error.

Audiovisual Aids

Lantern and microscopic slides for illustrating the relationship of indices values to the appearance of the red cells.

Laboratory Exercises

The objective of this section is to broaden the student's view of the stained slide and its relationship to known and calculated values.

Equipment: Materials for performing complete blood counts in combination with a sedimentation rate.

Exercises: The student should do the determinations necessary to calculate red blood cell indices in combination with a sedimentation rate. He should calculate the values for red cell indices and state whether or not these results are reasonable according to the appearance on the smear. Slides may be demonstrated for which they must estimate the MCV and MCH values from the appearance of the red cells on the smear.

Study Questions

1. On each of the following calculate: (a) MCV; (b) MCH; (c) MCHC. State whether or not the results you obtained would be possible (technically), or should one of the results used be repeated incorrectly. If the situation seems technically correct, describe the appearance of the red blood cells, using correct terminology. (To instructor—Give several results for calculation. Some of the problems should be construed so that the MCHC, for example, will be too high, and the student should recognize that there is a technical error.)
2. Erythrocytes with MCV and MCHC less than normal would be described as ________________.
3. If a patient has a marked anemia you would expect the ESR to be ________.
4. When diluting a patient's blood in a white pipette, you noticed that a very dark brown color appeared. The corresponding white cell count was 18,000 cu mm. The other laboratory data were:

Hgb.: 10.8 gm/100 ml.
Hct.: 49 percent.
RBC: 4,800,000 cu mm
Retic count: 1.0 percent.

Give your impression of this situation and explain. (Do *not* try to diagnose the patient; is the situation probably correct *technically*?)

K. Anemias:

1. General definition of anemia:
   a. Its role as a secondary symptom.
   b. The hemopoietic equilibrium.
2. Physiological factors affecting hematological picture in anemia:

a. Reflection on health by composition of peripheral blood:
    (1) In health the balance between the formation and destruction of erythrocytes and hemoglobin shown by constant level of these two substances in the peripheral blood.
    (2) Upset of balance results in anemia—physiological manifestations indicate which phase of the balanced mechanism is at fault:
        (a) Characterizations of excessive red cell destruction.
        (b) Characterizations of decrease in formation of red cells.
        (c) Qualitative changes in decreased hemoglobin and red cell contents—appearance of immature red cells in blood—reticulocytes or nucleated red cells.

b. Clinical signs of anemia:
    (1) Most physical signs due to general decrease in oxygen-carrying capacity of the blood.
    (2) Physical signs more dependent upon underlying disease processes and their complications.
    (3) General and progressive complaints.

3. Morphological classification of anemias; comparison of macrocytic and normocytic and microcytic anemias by study of specific cases with slides.

Audiovisual aids and demonstration material

Select an anemia from the macrocytic, microcytic, and normocytic groups; discuss and contrast it in detail with other members of the same group and with those in the other two groups. For example, use pernicious anemia (macrocytic), iron deficiency (microcytic), and hemolytic anemic (normocytic). Be sure to contrast the etiology and appearance of cells in thalassemia and iron deficiency anemias. Have the students make a chart to include the following categories for each one of the three anemias: hemoglobin, red count, white count, platelets, reticulocyte count, MCV, MCH, MCHC, and description of slide appearance. This can be done together so that every student will have the assistance of the instructor when finding the value for the active disease and after therapy, for example.

Laboratory Exercises

By this time in the training program, the exercises should include CBC's, red cell constants, platelet counts, and sedimentation rates. Vary the combination so that the student has an opportunity to make decisions as to procedure and adequacy of his own work.

Study Questions

1. Differentiate between absolute and relative leukocytosis.
2. A patient has an anemia, and after a thorough work-up, the physician prescribes iron as the preferred treatment. Give a probable range for the values of the following tests before treatment is started: hemoglobin, red count, MCV, MCH, MCHC, hematocrit, reticulocyte count. Which one of the above factors would change earliest after the treatment started? Why? This type of anemia is called ________. What would be the most probable cause or causes for this type of anemia in the following situations?
    a. Infant or young woman.
    b. Woman 28 years of age.
    c. Man over 40 years of age.
3. In what type of anemia is the spherocyte or spheroidocyte a characteristic finding? What are the two main types of this anemia (in regard to the cell defect and way in which the disease is acquired)? What are the probable values for the following in this situation: MCV, MCH, MCHC? Why would it be difficult to state a definite or probable value for the WBC, Hgb, and RBC in regard to this disease? A certain factor in the physical make-up of the spherocyte is used as the basis for the red blood cell osmotic fragility test. What is this factor?
4. Continue this type of thorough study question to include all the areas covered in this unit and in previous units to allow the student a complete review.

Unit VI Clinical Chemistry

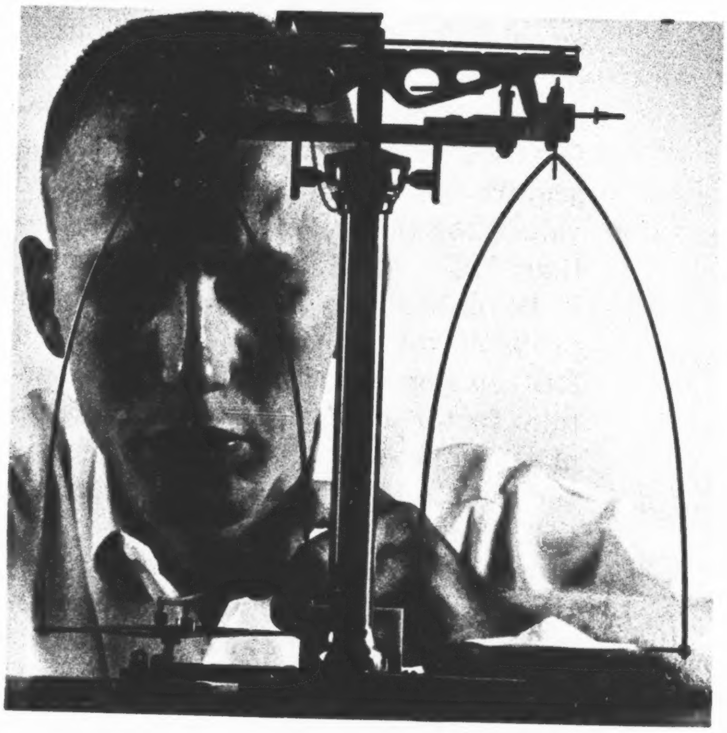
Suggested Time: 10 weeks; 400 hours.

Introduction

The basic instruction in chemistry is divided into eleven sections which should take a minimum of thirty discussion periods to cover adequately. Some instructors may prefer to discuss glucose immediately after protein-free filtrates and also keep all tests employing a protein-free filtrate in one group, then branch out to other analyses. The order of presentation is thus flexible. Suggested references for the instructor are given for each section, and special mention is made of suggested references for the student which may be especially applicable.

Suggestions are given for student practice exercises to be followed during the presentation of each section. These exercises may require varying lengths of time for individual students, but the instructor should see that each student is capable of performing each exercise adequately before allowing him to proceed.

Study questions are presented at the end of each section to enable the student to judge his own knowledge of the material which has been presented. Instructors are urged to supplement or change these questions to fit their own particular teaching situation.



Unit content

A. Protein-Free Filtrates.
B. Use of Standard Solutions and Quality Control in the Clinical Laboratory.
C. Non-protein Nitrogen.
D. Urea Nitrogen.
E. Protein (Total and Albumin Fractionation).
F. Glucose.
G. Amylase.
H. Bilirubin.
I. Cephalin—Cholesterol Flocculation Test.
J. Uric Acid.
K. Creatinine.

Bibliography

Annino, Joseph S., *Clinical Chemistry Principles and Procedures*, 3d ed., Boston, Little Brown and Co., 1964.

Davidsohn, I., and Wells, B. B., *Clinical Diagnosis by Laboratory Methods*, 13th ed., Philadelphia, W. B. Saunders Co., 1962.

Freier, E. F., and Rausch, V. L., "Quality Control in Clinical Chemistry," *American Journal of Medical Technology*, 24:195, 1958.

Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 13th ed., New York, Blakeston and Co., 1954.

Henry, Richard J., *Clinical Chemistry, Principles and Technics*, New York, Hoeber Med. Div., Harper and Row, 1964.

Hepler, Opal E., *Manual of Clinical Laboratory Methods*, 4th ed., Springfield, Ill., Charles C. Thomas, 1958.

Hoffman, W. S., *The Biochemistry of Clinical Medicine*, 2nd ed., Chicago, Yearbook Publishers, 1959.

Levinson, S. A., and MacFate, R. P., *Clinical Laboratory Diagnosis*, 6th ed., Philadelphia, Lea and Febiger, 1961.

MacFate, Robert P., *Introduction to the Clinical Laboratory*, Chicago, Yearbook Publishers, 1961.

Mollison, P. L., *Blood Transfusion in Clinical Medicine*, 3rd ed., Philadelphia, F. A. Davis Co., 1961.

Page, Lot B., and Culver, Perry J., *A Syllabus of Laboratory Examinations in Clinical Diagnosis*, Cambridge, Mass., Harvard University Press, 1961.

Peters, John P., and VanSlyke, D. D., *Quanti-*

tative Clinical Chemistry, Vol. II, Baltimore, Williams and Wilkins, 1956.

Reiner, M., ed., *Standard Methods of Clinical Chemistry*, Vol. I, II, III, New York, Academic Press, 1953, 1958, 1961.

Simmons, J. S., and Gentzkow, C. J., *Medical and Public Health Laboratory Methods*, 5th ed., Philadelphia, Lea and Febiger, 1955.

Unit outline

A. Protein-Free Filtrates:

1. Principle of the protein-free filtrate.
2. Purpose of the protein-free filtrate.
3. Methods of making protein-free filtrates:
   a. Folin-Wu filtrate.
   b. Haden modification of the Folin-Wu filtrate.

Demonstrations

The actual procedures used in the laboratory should be demonstrated as discussed. Stress should be placed on the type and cleanliness of the glassware, care of the reagents, proper technique, and sources of error. Improperly prepared filtrates will be helpful to show the student the result of improper procedure.

Laboratory Exercises

Equipment needed: All usual equipment and reagents for preparation of the protein-free filtrate. Tubes of sample unknowns in the form of whole blood, serum, or plasma.

Each student should set up duplicate filtrates, using each of the specimens supplied. Stress must be placed upon good technique and sources of error. A report sheet should be kept. The following suggested sample is given:

Specimen	Type of specimen	Appearance of specimen (normal lipemic, etc.)	Appearance of filtrate (clear, colorless, colored, cloudy)
	Whole blood		
	Serum		
	Plasma		

The instructor should check the technique of each student and should have filtrates checked carefully before they are considered satisfactory. The student should repeat this exercise until the instructor feels that his results are reliable.

Study Questions

1. Name the three agents for precipitating proteins and give an example of each.
2. In equation form, state the reaction which takes place when the Haden modification of the Folin-Wu method is used to precipitate the protein.
3. Before most chemical procedures are carried out, what initial step must be performed to eliminate possible interference?

B. Use of Standard Solutions and Quality Control in the Clinical Laboratory:

1. Need for reliability of results in the laboratory:
   a. Accuracy and precision of determinations parallel reliability:
      (1) One of the main difficulties lies in the fact that no method of analysis gives exactly the same result each time it is repeated.
      (2) The second main difficulty lies in the fact that we can use only a small amount and must consider it representative of the many thousands of milliliters of blood in the body.
   b. The laboratory itself, as well as the physician, must be assured that the results are accurate.
2. Methods of control:
   a. Standard solutions:
      (1) Definition and purpose.
      (2) Preparation of the standard and reading of curve.
      (3) Plotting of the standard curve.
      (4) Value of the often checked curves over the permanently calibrated curves.
      (5) Use of the reagent blank.
   b. Control solutions:
      (1) Types of control solutions:
         (a) Pooled serum from the laboratory—how it is used.
         (b) Commercially prepared—how it is used.
   c. The use of duplicate determinations.
   d. The use of recovery solutions.

Suggested reading assignments for students

Frier and Rausch, p. 195.
MacFate, pp. 284–293.

Demonstrations

The student should practice construction of standard graphs and the reading of concentrations from them.

Study Questions

1. What are standard solutions and why do we use them?
2. What is the relationship between percent transmission and concentrations?
3. Draw an example of the semi-logarithmic graph paper and describe each of the scales thoroughly.
4. Why is a blank tube included with each determination?
5. What is meant by the "origin" on the graph paper?
6. Name four means by which a laboratory can control the reliability of the results it turns out.

C. Non-protein Nitrogen*:

1. Nitrogen-containing substances:
   a. Protein nitrogen.
   b. Non-protein nitrogen:
      (1) Forms.
      (2) Importance of use of protein-free filtrate.
      (3) Composition of NPN.
      (4) Significance of estimation of NPN and importance in evaluating renal function.
      (5) Normal NPN values.
2. Methods used in quantitative estimations of NPN:
   a. Micro Kjeldahl.
   b. Modification of classic Kjeldahl method:
      (1) Principle.
      (2) Importance of nitrogen and ammonia free glassware and reagents.
      (3) Basic change in all modifications is a change in the digestion mixture.
         (a) Ammonia freed, distilled into acid and titrated.
         (b) Ammonia is determined colorimetrically after Nessler's solution is added.
         (c) Ammonia nitrogen is set free as a gas which is then measured.
   c. Method of Folin-Wu, brief discussion.
   d. Method of Koch-McMeekin, brief discussion.
   e. Method of Bock and Benedict.

Suggested reading assignments for students

Annino, pp. 144–153, 158–160.
Hawk, Oser, and Summerson, pp. 545–548.
Page and Culver, pp. 342–345.

Demonstrations

It is wise here to demonstrate the actual procedure to be used in the laboratory exercise. The step-wise procedure for the method of determining NPN used at your hospital should be described. As each step is described, the proper apparatus, reagents, and techniques should be demonstrated. The same type of demonstration applies to the Kjeldahl method.

Laboratory Exercises

The students should set up protein-free filtrates and, utilizing a Kjeldahl method, estimate NPN in a given sample. A report sheet should be provided for each student where the data accumulated during the procedure can be recorded. The entire procedure should be carried out under the supervision of the instructor. In recovery tests, a recovery of 90–110 percent is necessary to be within range. A standard solution should be used to check on the distillation process and must be within an acceptable range. The student should repeat this exercise until the instructor feels that he is proficient at it and his results are reliable.

Study Questions

1. Name three of the forms in which NPN can be found in the body.
2. Which of these forms is the chief determinant of the NPN value?
3. What is the name of the general method used for determination of NPN value?

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* This procedure has been considered outdated by many and has been replaced by the BUN test (see next section). If the NPN is not used in your laboratory, it need not be taught to the student.

4. What is the normal range for NPN in whole blood? In serum or plasma?
5. What do high NPN values signify?

D. Urea Nitrogen:

1. The place of urea in body function:
    - a. Relation to protein metabolism.
    - b. Concentration in blood is index of ability of kidney to remove waste material from blood.
    - c. Meaning of increased urea values in blood.
2. Methods to determine urea vary in use (suggests that the ideal method has not yet been found):
    - a. Anticoagulated blood, serum, or plasma can be used.
    - b. Most used and reliable methods use enzyme urease:
        - (1) Affected by time, temperature pH, concentration of substrate (urea), and by the presence of enzymatic poisons or inhibitors.
        - (2) Brief description of enzymes in general.
    - c. Most frequently used methods require urease added to whole blood.
    - d. Nesslerization of a portion of protein-free filtrate commonly used.

Suggested reading assignments for students

Annino, pp. 154–168.
Page and Culver, pp. 342–345.

Demonstrations

The actual procedure to be used in the laboratory should be demonstrated. The proper pipetting of whole blood using an Ostwald pipette, incubation procedure, preparation of protein-free filtrates, color development, (Nesslerization), and colorimetric analysis should be included.

Laboratory Exercises

The students should perform the examination under supervision, preparing a standard curve, running controls, recovery tests, and determination of unknown in duplicate. The proper use of recovery solutions and the reason for their use should be emphasized. A chart should be prepared showing galvanometer readings, concentration of standards, and calculations to reach answer in milligrams percent. The standard graph should be turned in with this chart. The student should repeat this exercise until the instructor feels that he is proficient at it and that his results are reliable. Acceptable recovery range should be 90–110 percent.

Study Questions

1. An increase in the level of urea nitrogen in the blood is indicative of disease or malfunction of what organ in the body?
2. In the direct Nesslerization method ....is converted to .... by the enzyme.
3. What are the normal values for blood urea nitrogen?
4. For what reason is a recovery solution used in this BUN procedure?
5. Why should sodium fluoride never be used as an anticoagulant in a BUN procedure?

E. Protein (Total and Albumin Fractionation):

1. Place of protein in the life processes:
    - a. Protein substances.
    - b. Protein deficiency and how it affects the body.
    - c. Plasma proteins:
        - (1) Albumin—significance of abnormal values.
        - (2) Globulin—significance of abnormal values; fractions of globulins.
    - d. Synthesis of protein.
    - e. Significance of abnormal protein values in general.
2. Methods to determine plasma proteins:
    - a. Total protein content or the fractionation into component parts (such as albumin and globulin) may be determined.
        - (1) Total protein methods used:
            - (a) Kjeldahl method—theory and procedure described.
            - (b) Biuret method—theory and procedure described.
        - (2) Fractionation (Globulin is precipitated and removed and al-

        bumin content determined, globulin content calculated by subtracting albumin value from total protein value).
    (3) Albumin and globulin determination:
        (a) Modification of Howe method, a salting-out process.
        (b) Theory and procedure described.
    (4) Electrophoresis.
    (5) Use of standard protein solutions.

Suggested reading assignments for students

Annino, pp. 184–194.
Page and Culver, pp. 234–240.

Demonstrations

The actual procedure used in the laboratory should be demonstrated, stressing technique and sources of error.

Laboratory Exercises

The procedure for total protein and the procedure for albumin fractionation and analysis should be performed, with standards, controls and unknowns. Charts should be prepared for the student to record all readings and determinations. The instructor should check individual technique and have the student repeat the exercise until the instructor feels that the student is proficient at it and that the results are reliable.

Study Questions

1. Proteins are made up of combinations of units called what?
2. Serum protein are divided into what two major groups?
3. What is the biuret reaction?
4. Proteins may be separated by what two major methods?
5. Name some pathological conditions in which the protein concentration is of importance to the doctor.
6. What is the normal range for total proteins, albumin, and globulin?

F. Glucose:

1. Place of carbohydrates in body function.
2. Glucose:
    a. Mechanism controlling concentration.
    b. Time to determine glucose:
        (1) Fasting specimen.
        (2) Post-prandial.
    c. Value of whole blood, serum, and plasma.
    d. Glycolysis.
        (1) Importance of speed in preparation of protein-free filtrate.
        (2) Use of refrigeration and/or sodium fluoride.
    e. Normal ranges.
    f. Maintenance of normal values by the body.
        (1) Physiology of increased values.
        (2) Physiology of decreased values.
    g. Methods—Most make use of fact that glucose contains an aldehyde group with reducing abilities:
        (1) Folin-Wu—classic method but measures reducing substances other than glucose.
        (2) Benedict—more specific but color is not stable.
        (3) Somogyi—measures true glucose.
        (4) Nelson-Somogyi—most reliable method.
    h. Importance of special tubes in performing glucose determinations.
    i. Demonstration and discussion of Nelson-Somogyi method.

Suggested reading assignments for students

Annino, pp. 133–143.
Davidsohn and Wells, pp. 415–422.

Demonstrations

The actual procedures to be used in the laboratory should be demonstrated. The proper pipetting of blood using an Ostwold pipette, the Folin-Wu tube, preparation of filtrates, color development, and colorimetric analysis should be demonstrated.

Laboratory Exercises

The student should prepare test runs, using the procedure demonstrated. A standard curve should run, control samples and unknowns—all in duplicates. Charts should be prepared giving all information and calculations. The instructor should observe individual technique and should have the student repeat the examinations until the instructor feels that the student is proficient at it and that the results are reliable.

Study Questions

1. Which anticoagulant is best for doing blood glucose determinations?
2. To precipitate the protein in the Nelson-Somogyi method, one uses ________________ and ________________.
3. Upon what does the ability of glucose to reduce copper depend?
4. What is the purpose of using the special Folin-Wu sugar tube?
5. What are two conditions necessary for the reduction by glucose to take place?
6. Write the reaction which occurs during the reduction process.

G. Amylase:

Understanding the determination for amylase and, indeed, all enzyme methods requires a background in organic and physiological chemistry. The laboratory assistant student does not have this background. No doubt he will have the mechanical ability to perform the test but will be so limited in understanding what he is doing that the chances of error are greatly increased. If the individual training program requires that the student learn to do amylase, the instructor will have to prepare lectures to fit the average ability to learn in each class. A lecture introducing an enzyme procedure would have to include material concerning structure, classification, mode of action, factors affecting activity, substrate, activation, inhibition, specificity, methods of determining activity, and a definition of the units in which enzyme concentration is usually expressed, i.e., degrees of enzyme activity. If it is felt that this procedure should be included, material to help in planning the lecture and the laboratory exercises may be found in clinical chemistry texts and references, such as *Clinical Chemistry Principles and Procedures* by J. S. Annino, op. cit., and *A Syllabus of Laboratory Examinations in Clinical Diagnosis* by Page and Culver, op. cit.

H. Bilirubin:

1. Physiology of formation:
   a. Structure of bilirubin.
   b. Production of bilirubin.
2. Methods of determination:
   a. Van den Bergh reaction common basis:
      (1) Indirect bilirubin.
      (2) Direct bilirubin.
   b. Malloy and Evelyn method; modifications.
3. Abnormal bilirubin values found in jaundice:
   a. Physical appearance of patient.
   b. Types of jaundice:
      (1) Retentive jaundice:
         (a) Physiology.
         (b) Findings.
         (c) Conditions in which it exists.
      (2) Regurgitative jaundice:
         (a) Physiology.
         (b) Findings.
         (c) Conditions in which it occurs.

Suggested reading assignments for students

Hoffman, pp. 326–339.
Mollison, Chapters 16 and 17.
Page and Culver, pp. 396–411.

Demonstrations

It is wise to demonstrate the actual procedure used in the laboratory. As each step is described, the proper apparatus, reagents, and techniques should be demonstrated. Special emphasis should be given to use of fresh unhemolyzed serum, technique for mixing cuvettes, importance of absence of bubbles when reading tubes, technique used in timing 1-minute specimens, and preparation of fresh Ehrlich's diazo color reagent.

Laboratory Exercises

The student should practice the procedure demonstrated, including controls in duplicate and at least one unknown in duplicate. He should be watched quite carefully the first time and will probably need help in timing the 1-minute determinations. The student should repeat this exercise until the instructor feels that he is proficient at it and that his results are reliable. A chart should be used to record all work.

Study Questions

1. Why must the Ehrlich's reagent be fresh with each series of bilirubin determinations?
2. Describe the van den Bergh reaction. Also discuss the difference between the direct and indirect reactions.
3. Why must you use unhemolyzed serum or plasma in this procedure?
4. What should you do if your specimen reads less than 10 percent transmission? Why?
5. Using Page and Culver, pp. 400–407, as a guide, discuss the five major types of jaundice, their cause, and laboratory findings. Include a distinction between retentive and regurgitative types of jaundice and their respective laboratory findings.

I. Cephalin-Cholesterol Flocculation Test:

1. Principles of flocculation tests in general:
   - a. Screening tests based upon variations in amount and type of protein produced.
   - b. Mechanism of flocculation and turbidity tests.
     - (1) Relation of electrolytes to lyophilic colloidal particles of protein.
     - (2) Various tests were designed to cause proteins in pathological serums to become unstable and precipitate out of solution, while normal proteins remain stable and, therefore, remain in solution.
   - c. Three general types of flocculation and turbidity tests:
     - (1) Cephalin-cholesterol flocculation—reagent charged colloidal suspension which is flocculated out of solution by a change of the distribution of proteins in the serum.
     - (2) Thymol turbidity—involves precipitation of certain abnormal protein fractions by compounds which contain phenolic linkages or strongly polar compounds like water and alcohol.
     - (3) Zinc sulfate turbidity—involves interaction between abnormal protein and a divalent ion (Zn++), resulting in precipitation of the proteins.
   - d. Cephalin cholesterol flocculation accepted as an accurate index of the active disturbance of liver parenchyma:
     - (1) Most valuable in differential diagnosis of chronic and acute disease if combined with alkaline phosphatase.
     - (2) Generally lacks specificity and will be altered in a number of clinical conditions not related to liver parenchyma.

Suggested reading assignments for students

Annino, pp. 230–232.
Page and Culver, pp. 419–422.

Demonstrations

It is wise to demonstrate the actual procedure used in the laboratory. As each step is described, the proper apparatus, reagents, and techniques should be demonstrated. If possible, have examples of 1+, 2+, 3+, and 4+ reactions on hand for demonstration. Be certain the students understand how to grade the results since this is rather subjective.

Laboratory Exercises

The student should practice the procedure as described. Stress to student potential sources of error, such as exposure to light, increased reaction temperature, inadequate preparation of the reagent, age of the serum, and contamination of glassware with traces of acid. Students should repeat this exercise until considered proficient and reliable.

Study Questions

1. Why are the flocculation and turbidity tests termed empirical tests of liver damage or disease rather than liver function tests?
2. Most of the flocculation and turbidity tests are measuring an increase of what portion of the serum?
3. Explain in your own words the general mechanism behind the flocculation and turbidity tests.

4. Describe the principle behind the three main types of flocculation and turbidity tests as described in Page and Culver.
5. Discuss the clinical application of the cephalin-cholesterol flocculation test and flocculation tests in general.

J. Uric Acid:

1. General discussion of uric acid:
    a. In urine, result of protein catabolism; in blood, result of protein metabolism.
    b. Normal values.
    c. Significance of elevated values.
2. Methods of analysis:
    a. Several methods use cyanide in conjunction with phosphotungstate color reagent:
        (1) Correct amount of lithium salts reduces tendency of cyanide to produce turbidity.
        (2) Urea will avoid turbidity as well as stabilize the cyanide so most procedures based on the phosphotungstate reaction are made more specific by sodium cyanide-urea solution.
        (3) Blauch-Koch method makes it even more specific by use of enzyme uricase:
            (a) Normal values.
            (b) Interfering substances.
    b. Sodium silicate and sodium carbonate have been substituted for cyanide in some tests.
        (1) Discussion of procedure for method using sodium silicate
        (2) Discussion of procedure for method using sodium carbonate.

Suggested reading assignments for students

Annino, pp. 179–183.
Davidsohn and Wells, p. 23 and pp. 451–452.

Demonstrations

It is wise to demonstrate each step of the actual laboratory procedure as it is introduced to the student.

Laboratory Exercises

The student should practice the procedure as described, including controls and recoveries in duplicate and at least one specimen in duplicate. He should be watched carefully the first time and will probably need help in reading the tubes in the colorimeter the first time, since they are timed. Reports of results should be made on a chart form. As before, the student should become proficient in this exercise before proceeding to the next section.

Study Questions

1. Upon what reaction are most uric acid reactions based?
2. What are the three basic modifications of this reaction used in uric acid procedures, and what is the prime reason for these modifications?
3. Which uric acid procedure described is the most specific for uric acid and should be used as a reference method? Why?

K. Creatinine:

1. Comparison of creatine and creatinine:
    a. Structurally very similar.
    b. Since creatinine is waste product of creatine, concentration of former is proportional to latter present in body.
2. Creatinine:
    a. Amount of creatinine excreted daily is constant since it comes from the amount of creatine in the skeletal mass.
    b. Creatinine level in serum is proportional to glomerular filtration rate:
        (1) Main reason for changed creatinine concentration in blood is impaired kidney function.
        (2) Converse of (1) is not true—normal creatinine concentration does not necessarily indicate good renal function.
3. Method of analysis most based on Jaffe reaction: Interfering substances can make it non-specific for creatinine.
    a. In red blood cells, 30–50 percent of Jaffe reaction due to creatinine.
    b. In serum, plasma or protein-free filtrate, 80–100 percent of reaction due to creatinine.

Suggested reading assignments for students

Annino, pp. 173–178.
Page and Culver, pp. 348–351.

Demonstrations

Demonstrate the procedure used in the laboratory, stressing step-by-step the apparatus, technique, reagents, and sources of error.

Laboratory Exercises

The student should practice the creatinine determination including a black, standard curve, control in duplicate, and unknowns in duplicate. Reports should be made on a prepared chart form; the student should become proficient in the determination of this substance.

Study Questions

1. Describe the Jaffe reaction.
2. Creatinine is a waste product derived from what body substance? Of what use is this substance in the body?
3. Explain fully why the daily amount of creatinine excreted by any one individual is so remarkably constant from day to day.
4. In what general condition or conditions is the urine creatinine concentration increased and decreased?
5. Increased serum creatinine is usually an indication of dysfunction of what organ? Why?

Unit VII Blood Banking

Suggested Time: 6 weeks; 240 hours.

Introduction

Heavy and direct responsibility of blood bank work:

1. Need for exact technique and avoidance of short cuts.
2. Need for elaborate safeguards and seemingly repetitive checks.
3. Need for legible, permanent, and accurate complete and identified records of all work in the blood bank.

Unit content

A. Blood Donors.
B. Blood Collection.
C. ABO Blood Groups.
D. Serum Grouping for ABO.
E. Rh Factors.
F. Other Blood Groups.
G. Anti-human Globin Test (Coombs Test).
H. Compatibility Testing or Crossmatching.
I. Processing, Storage, Issue of Donor Blood.
J. Blood Bank Records.

Bibliography

American Association of Blood Banks, *Technical Methods and Procedures,* Chicago, The Association, 1962.

DeGowin, Elmer L., Hardin, R. C., and Alsever, J. B., *Blood Transfusion,* Philadelphia, W. B. Saunders Co., 1949.

Dunsford, Ivor, and Bowley, Christopher, *Techniques in Blood Grouping,* Springfield, Ill., Charles C. Thomas, 1955.

Mollison, P. L., *Blood Transfusion in Clinical Medicine,* 2d ed., Springfield, Ill., Charles C. Thomas, 1956.

Race, R. R. and Sanger, Ruth, *Blood Groups in Man, Philadelphia,* F. A. Davis Co., 1962.

Stratton, F., and Renton, P. H., *Practical Blood Grouping,* Springfield, Ill., Charles C. Thomas, 1958.

U.S. Public Health Service, *Biological Products,* Public Health Service Regulations, Title 42, Part 73, Washington, D.C., 1962.

Wiener, Alexander S., *Blood Groups and Transfusion,* Springfield, Ill., Charles C. Thomas, 1943.

Available Booklets

Hyland Laboratories, *Hyland Reference Manual of Immunohematology,* The Laboratories, 4501 Colorado Blvd., Los Angeles 39, Calif.

Ortho Diagnostic Division, *Blood Group Antigens and Antibodies as Applied to Blood Transfusion,* Raritan, N.J., Ortho Pharmaceutical Corp., 1960.

U.S. Public Health Service, *Laboratory Procedures for Modern Syphilis Serology,* Communicable Disease Center, Atlanta, Ga., 1961.

Journals of interest to Blood Bank personnel

Blood.
Journal of Laboratory & Clinical Medicine.
Immunology.
Transfusion.
Vox Sanguinis.

Unit outline

A. Blood Donors:

1. Registration of donor:
   - a. Name, address, etc.
   - b. Medical history:
     - (1) Accurate medical history—protection to both donor and recipient.
     - (2) Discussion of each question and why it is asked; emphasis on hepatitis.
     - (3) What disqualifies a donor; what to refer to M.D. for decision.
2. Physical examination:
   - a. Demonstration and discussion of method for determining hemoglobin concentration and the acceptable concentration in a donor.
   - b. Demonstration and discussion of blood pressure determination and acceptable range of systolic and diastolic pressures.
   - c. Acceptable pulse rate, temperature range, and body weight.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 1–4, 80, 83–85.

Laboratory Exercises

Equipment needed:

- a. Sphygmomanometer and stethoscope.
- b. Thermometers and cleaning solutions.
- c. Sterile disposable or sterilized lancets.
- d. Appropriate equipment for hemoglobin determination.

1. Practice taking medical histories that are complete, permanent, and identified.
2. Have demonstration and practice in hemoglobin determination; in taking blood pressures; in placing, timing and reading of thermometers.

Study Questions

1. What diseases are transmissible by blood transfusion?
2. How often may a donor give blood?
3. What is the minimum level of hemoglobin a donor must have?
4. Why are donors with a history of jaundice rejected as donors for whole blood?
5. If a donor told you he had a heart condition, what would you do?

B. Blood Collection:

A combined lecture and demonstration is recommended procedure.

1. Preparation of donor's arm to give maximum assurance of a sterile container of blood:
   - a. Solutions to be used, such as green soap, 70 percent alcohol, and 2 percent iodine (dried).
   - b. Technique of cleansing with sterile cotton gauze used with forceps kept in antiseptic solution or with sterile cotton-tipped applicator sticks.
2. Identification of donor and confirmation that donor number on the history card, the pilot and laboratory tubes, and the blood container is the same.
3. Preparation of blood container and donor set for (a) gravity bleeding or (b) vacuum bleeding.
4. Phlebotomy.
5. Care of the donor.
6. Donor reactions—procedure.
7. Care of the unit of blood.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 6–8, 75–76, 85.
DeGowin et al, pp. 236–245.
PHS, *Biological Products*, pp. 51–52.

Laboratory Exercises

1. Review techniques of donor arm preparation; observe, then perform a preparation.
2. Review checking of donor identification on donor card, on pilot laboratory tubes, on blood container; assist by doing it.
3. Observe a phlebotomy; perform one under close supervision.
4. Place units of blood in proper refrigerator, laboratory tubes in their proper place, and filled-out donor card in its place.
5. Observe and learn to care for donor with a reaction.

Study Questions

1. What is the purpose of a thorough donor arm preparation?
2. When are pilot tubes attached to the blood container? When is the number attached?
3. When are the pilot tubes filled?
4. If you are not able to get the needle into the donor's vein on one arm, what do you do?
5. If during the drawing a donor complains that he feels ill, what would you do?

C. ABO Blood Groups:

1. Discovery of human blood groups A, B, and O by Landsteiner in 1900.
2. Group AB discovered by von Decastello and Sturli in 1902.
3. M, N, and P by Landsteiner and Levine 1927.
4. Rh by Landsteiner and Wiener 1940.
5. Discussion of the four blood groups as antigens and antibodies, agglutinogens and agglutinins, iso-agglutinins.
6. Brief discussion of inheritance of blood groups.
7. Incidence of blood groups in United States.

Suggested reading assignments for students

DeGowin et al, pp. 52–63.
Dunsford & Bowley, pp. 3–7.
Mollison, pp. 176–182.
Race and Sanger, pp. 9–49.
Stratton & Renton, pp. 107–112.
Wiener, pp. 7–18.
AABB, *Technical Methods*, pp. 11–12.
Hyland Reference Manual.
Ortho, *Blood Group Antigens.*

Audiovisual Aids

Blood Grouping, color, sound, 21 min., Film Library, Imperial Chemical Industries, 488 Madison Ave., New York 22, N.Y.

Demonstrations

Identification of slides or tubes and performance of direct grouping using known antiserums by slide and/or tube technique.

Introduction to Laboratory Method

1. Use of a known anti-A and anti-B serum on unknown blood samples of donors and patients and on known controls.
2. Equipment needed:
   a. Clean glass slides and/or tubes 12 x 75 or 13 x 75 mm.
   b. Applicator sticks or disposable pipettes.
   c. Centrifuge.
   d. Interval timer.
3. Reagents: Commercial anti-A and anti-B serums.
4. Specimens used:
   a. Samples of whole blood of unknown group.
   b. Samples of whole blood of known group A, B, AB, and O for examples and controls.
5. Method:
   a. Read and follow carefully the directions for use that come with the antiserums.
   b. If clotted blood used, show rimming and centrifuging of samples.
6. Sources of error:
   a. Too heavy a cell suspension may lead to absorption of antiserum without agglutination.
   b. Tests should be observed for specified time since a weak antigen may react slowly.
   c. Presence of clots may be interpreted as false positive.
   d. Heavy bacterial contamination can cause error.
   e. Reaction temperature higher than room temperatures can cause error.

Laboratory Exercises

1. Review antigen-antibody reactions and demonstrate direct ABO grouping using samples of known and unknown ABO groups.
2. Introduce student to the laboratory sheet for recording results; results must be permanent, dated, and signed.
3. Have students perform tests.

Study Questions

1. What blood groups did Landsteiner discover? When?

2. Describe an antigen and an antibody.
3. Describe briefly how blood groups are inherited.
4. What is the incidence of the blood groups in the United States?
5. Is heat used for ABO tests?
6. What is a hemolysin?
7. What are some possible sources of error in doing direct grouping tests?

D. Serum Grouping for ABO:

1. Review Landsteiner discovery.
2. Review antigens and antibodies:
   a. Naturally occurring anti-A and anti-B.
   b. Hemolysins.
   c. Immune antibodies.
   d. Rouleaux and cold agglutinins.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 12–13.
DeGowin et al., pp. 70–77.
Dunsford & Bowley, pp. 8–10, 13–18, 21–22.
Mollison, pp. 175, 182–201.
Ortho, pp. 10–11.
Race and Sanger, pp. 17–49.
Wiener, pp. 18–34.

Audiovisual Aids

Fundamentals of Human Blood Groups, filmstrip, color, 35 mm., and disc, 16 inch, 33⅓ rpm., 15 min., Communicable Disease Center, Atlanta, Ga.

Technics in Blood Grouping, Part I—Slide Techniques, and Part II—Test Tube Techniques, filmstrips, color, accompanied by L. P. (33⅓ rpm) record. Audio-Visual Library. ASMT Education & Research Fund, Inc., Hermann Prof, Bldg., Houston, Tex., 77025.

Demonstrations

1. Preparation of washed known A cells and B cells in an approximate 2 percent suspension of cells in saline.
2. Identification of tubes and distribution of serum or plasma.
3. Addition of cells to proper tubes, incubation and/or centrifugation, reading and recording results.
4. Comparison of direct and serum grouping results to determine blood group.
5. Procedure in the event the two methods do not agree as to blood group.

Introduction to Laboratory Method

1. Equipment: clean glass tubes or slides, test tube racks, disposable pipettes, centrifuge, glass marking pencils.
2. Reagents: known A_1 and B cells, normal saline.
3. Specimens used: specimens of known and unknown blood groups. (Note: It is possible to prepare a "rouleaux" serum by adding Dextran to a normal serum for demonstration and student practice purpose.)
4. Occurrence of rouleaux and auto or cold agglutinins in serum; methods for solving these two problems.
5. Sources of error:
   a. Dirty glassware or pipettes.
   b. Placing cells in wrong tube.
   c. Hemolysis read as a negative result.
   d. Lack of agglutinins in patient's serum.
   e. Presence of irregular antibodies in serum which react at room temperature.
   f. Bacteriogenic agglutination.

Study Questions

1. What general types of anti-A and anti-B are there?
2. What may cause disagreement between the direct group and the serum group of the same sample?
3. How do you determine the serum group of a sample which contains cold agglutinins?
4. How do you determine a serum group on a specimen which exhibits rouleaux formation?
5. Should both the cells and serum of an unknown sample be tested for ABO group?

E. Rh Factors:

1. Landsteiner and Wiener's discovery of the Rh_0 (D) factor in 1940:
   a. Percentage of Rh_0 (D) positive and negative persons in the U.S. population.
   b. Importance of this factor in blood trans-

fusion and in pregnancy. Formation of anti-Rh_0 (D) in Rh negatives and percent of irregular antibodies that are anti-Rh_0 (D).

  c. Other Rh-hr factors.
  d. Weak or intermediate Rh factors.
  e. Complete and incomplete Rh antisera.
2. Fisher-Race nomenclature.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 14–20.
DeGowin et al., pp. 79–88.
Dunsford & Bowley, pp. 19–61.
Hyland, pp. 36–45.
Mollison, pp. 202–219.
Ortho, pp. 18–25.
PHS, *Biological Products*, p. 52.
Race & Sanger, *Blood Groups in Man*, pp. 115–173.
Stratton & Renton, pp. 154–189.
Wiener, pp. 245–254.

Demonstrations

Performance of anti-Rh tests using complete and incomplete serums and the methods called for in the directions for use of each serum.

Introduction to Laboratory Method

1. Review of formation of anti-Rh antibodies and their thermal optimum; also potentiation by macromolecules in some instances.
2. Equipment: glass slides, warm view box, tubes and racks, waterbath, centrifuge, applicator sticks and/or Pasteur pipettes, interval timer.
3. Reagents: antiserums, saline, albumin.
4. Anticoagulated or clotted blood from donors and patients.
5. Directions for use that come with antiserums most important.
6. Controls:
   a. Test cells with added albumin.
   b. Known positive and known negative cells.
7. Technical factors and sources of error:
   a. Omission of antiserum from test.
   b. Insufficient time for reaction to occur.
   c. Use of too concentrated cell suspension.
   d. Use of outdated antiserum.
   e. Excessive amount of anticoagulant in sample.
   f. Rouleaux formation.
   g. Antibody coated cells.
   h. Plasma clots.
   i. Dirty glassware.
   j. Heavy bacterial contamination of specimen or antiserum.

Laboratory Exercises

1. Review Rh factors and formation of antibodies to them.
2. Demonstrate Rh testing and have student perform tests, using controls.
3. Have student learn how and where to record the results.

Study Questions

1. What is the most clinically important Rh factor? Why is it clinically important?
2. Why do we use controls?
3. What two types of serum are used in Rh testing?
4. For what Rh factors can we test?
5. Name at least five possible sources of error in Rh testing.

F. Other Blood Groups:

1. M, N, and P discovered in 1927 by Landsteiner and Levine, following injection of rabbits with human blood cells.
2. Antibodies to blood groups usually found as result of incompatible crossmatches, transfusion reactions, and hemolytic disease of the newborn.
3. List of blood groups in order of their discovery (Race & Sanger).
4. List of most clinically significant antigens other than ABO and Rh.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 20–22, 24–27.
Hyland *Reference Manual*, pp. 29–32, 34–35, 47–55.
Mollison, pp. 224–249.
Ortho, pp. 27–36.
Race and Sanger, pp. 66–112, 183–232.

Demonstrations

Demonstrations of testing for blood group antibodies may be given with emphasis on following directions for use of serum and the use of positive and negative controls.

Introduction to Laboratory Method

1. Equipment and specimens—those used in ABO and Rh testing.
2. Reagents—may be licensed antiserums or serums found in laboratory testing or crossmatching.
3. Method—may be the directions for use of the licensed serums or the temperature, medium, and time of reaction found best for the laboratory serum.
4. Known positive and negative control cells tested in parallel with the unknown sample assure reactivity of the antiserum.
5. Sources of error as in Rh and ABO.
6. Clinical application: If patient has an unusual antibody, donor bloods may be screened for the antigen before crossmatch.

Laboratory Exercises

1. Review lecture notes and reading.
2. Demonstrate setting up and reading tests and recording results.
3. Have students do performance and recording of tests.

Study Questions

1. What are the clinically important blood factors in this group? Why are they clinically important?
2. Why is it important to follow directions for use that come with an antiserum?
3. Why is it important to use positive and negative controls in testing for antigens such as Kell and Duffy?
4. Which blood group system is different from all other systems so far discovered?
5. What is a "public" antigen and what is a "private" antigen?

G. Anti-human Globulin Test (Coombs Test):
1. Explanation of antibodies as globulins:
   a. Not all antibody-coated cells agglutinate in test tube.
   b. Anti-human serum produced in animals —usually rabbit or goat—agglutinate antibody-coated cells.
2. Direct Coombs test:
   a. Principal uses:
      (1) Testing infant's cells in suspected cases of hemolytic disease of newborn.
      (2) Testing cells of patients suspected of having auto-immune hemolytic anemia.
      (3) Testing of patient cells following a transfusion reaction.
   b. Method for doing test.
3. Indirect Coombs test:
   a. Principal uses:
      (1) Testing serum of pregnant women for antibodies, using previously characterized red cells.
      (2) Testing serum of patients and donors for antibodies, using cells as above.
      (3) Crossmatching.
      (4) Antigen testing for D^u, Kell, and other rare blood groups.
   b. Method for doing test.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 22–24, 65.
Dunsford & Bowley, pp. 63–69.
Hyland *Reference Manual*, pp. 58–66.
Mollison, pp. 299–309.
Ortho, pp. 37–42.
Stratton & Renton, pp. 60–78.

Demonstrations

Demonstrations of both direct and indirect antiglobulin tests may be done.

Introduction to Laboratory Method

1. Equipment: tubes, racks, waterbath, centrifuge, and squeeze bottle for adding of saline.
2. Reagents: anti-human globulin serum, serum containing an antibody such as anti-Rh_0 (D).
3. Specimens of blood with Rh_0 (D) and Rh_0 (D) negative.
4. Controls of antibody-coated cells may be purchased or prepared in laboratory.
5. Technical factors: each lot of anti-human serum varies; read directions for use. Anti-human serum being used may not demonstrate all antibodies.

6. Sources of error:
   - a. Even exceedingly small trace of serum can inactivate the Coombs reagent. Thorough washing of cells before adding Coombs serum is essential.
   - b. Contaminating blood samples with Wharton's jelly may cause agglutination of cells.
   - c. Detergents or chemicals on glassware or in saline may cause agglutination.
   - d. Incubation time of cells and serum may be too brief to allow coating.

Laboratory Exercises

1. Review of lecture notes and reading.
2. Supervised performance of tests.
3. Procedure of recording test results.

Study Questions

1. What is the principle of the anti-human globulin test?
2. Why must the cells be washed thoroughly before the anti-human globulin is added?
3. When is the direct Coombs used?
4. What is the difference between the indirect Coombs test and direct test?
5. Are all Coombs serums just alike?
6. What are the sources of error which must be avoided in performing the anti-human globulin test?

H. Compatibility Testing or Crossmatching:

1. Purpose of crossmatching:
   - a. Testing compatibility of donor's blood with recipient's blood for transfusion.
   - b. Need for accuracy and care, avoidance of all shortcuts.
2. Types of crossmatch:
   - a. Major.
   - b. Minor.
3. Crossmatching procedures and indications for use of each:
   - a. Saline.
   - b. Albumin.
   - c. Coombs.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 29–33, 88–89.
DeGowin et al, pp. 182–190.
Dunsford & Bowley, pp. 70–71.
Hyland *Reference Manual*, pp. 71–75.
Mollison, pp. 310–318.
Ortho, pp. 48–53.
Stratton & Renton, pp. 208–237.

Audiovisual Aids

The Point of No Return, color, sound film, 20 min., Ortho Diag. Div., Raritan, N.J.

Introduction to Laboratory Method

1. Equipment: same as for other procedures, plus microscope for reading crossmatches.
2. Reagents: Anti-A, B, and Rh_0 (D), albumin, anti-human globulin serum, enzyme, saline.
3. Specimen used:
   - a. Patient specimen must be identified clearly and accurately, with name, hospital number, ward or room, sex, service, date.
   - b. Donor blood must be from a pilot tube which was attached to the blood container and filled at time of donation.
4. Control measures may include:
   - a. Group and type of each patient.
   - b. Use of coated cells in Coombs crossmatch tubes.
5. Technical factors:
   - a. Rouleaux formation.
   - b. Cold agglutinins.
   - c. Patient cells positive to direct Coombs test.
6. Source of error:
   - a. Not adding serum to cells.
   - b. Inadequate identification of crossmatch tubes.
   - c. Use of patient sample which is too old (especially to be avoided when patient is having many transfusions).
   - d. Too violent agitation of crossmatch tube when suspending cells after centrifugation.

Study Questions

1. Why does the blood bank insist on having full identification in a patient sample of blood?
2. When is the proper time to label the sample of the recipient's blood?

3. Why is it important to have fresh samples of blood for crossmatch when the patient is having many transfusions?
4. List the test which should be performed in crossmatching blood for transfusion when there is no emergency. Give the reason for performing each test.

I. Processing, Storage, and Issue of Donor Blood:

1. Processing techniques have been learned except for a serological test for syphilis, which may or may not be routinely performed in the blood bank; instruction in performance of such a test, if necessary.
2. Organization of processing techniques and proper recording of results.
3. Labeling of blood:
   a. Information on printed label.
   b. Donor number; group and type.
   c. Expiration date: how it is determined and why.
   d. Checks and rechecks used in blood bank to assure that each unit of blood is correctly labeled as to group and type.
4. Blood storage conditions:
   a. Optimum storage temperatures within a 2° C. range between 1°–6° C.
   b. Cleanliness of blood storage refrigerator and freedom from extraneous materials for safety of blood stored there.
   c. Frequently tested alarm system as a precaution.
   d. Records of storage temperatures by a recorder chart or by twice daily reading and recording from accurate thermometer in the refrigerator.
5. Daily inspection and inventory of blood on shelf ready for issue; units of blood inspected for outdating, clots, hemolysis, unusual color or cloudiness; inspection record kept.
6. Culturing a unit of blood a month after it has "expired" to check on technique used in drawing blood.
7. Issuing blood:
   a. Blood issued by a blood bank to another blood bank or hospital.
      (1) Distribution records should include what, when, where, and who.
      (2) Shipping container should be capable of maintaining temperature below 10° C. during period of shipment.
   b. Crossmatched blood issued by a blood bank for a patient.
      (1) Request for the blood must be signed by a responsible person, must include the name, hospital number, room or ward, sex, age, etc., of patient, and must be carefully checked against the information on the crossmatched blood.
      (2) Release of a unit for transfusion must have safeguard checks.

Suggested reading assignments for students

AABB, *Technical Methods*, pp. 59–62, 87.
CDC, *Laboratory Procedures for Modern Syphilis Serology.*
PHS, *Biological Products*, pp. 52–56.

Demonstrations

May include STS, labeling, blood inspection, culturing, and issuing.

Laboratory Exercise

The techniques under this section can be learned by observation and then performance. Special emphasis can be given to labeling and to release of crossmatched blood.

Study Questions

1. Why does citrated whole blood (human) have an expiration date of 21 days?
2. Why is a serological test for syphilis done on blood for transfusion?
3. Why is blood stored at temperatures below 10° C.?
4. Why should a request for blood for transfusion to a patient include more than the name of the patient for whom it is intended?
5. Why should blood stored for issue be inspected?

J. Blood Bank Records:

1. Blood bank legally liable for negligence in its operation; inadequate and poorly kept records do not help in a court of law:
    a. Some 85 percent of errors in transfusion practice are clerical in nature rather than technical. For patient's safety, keep all records fully so that planned checks against error can operate. Copying of information should be kept to a minimum.
    b. Record forms vary, but records should be made concurrently with the performance of each procedure. They should be legible, indelible, initialed, and dated.
2. *Technical Methods and Procedures* of the AABB lists the kinds of records to be kept:
    a. Donor records, including donor reactions.
    b. Transfusion request records.
    c. Transfusion release records.
    d. Record of transfusion.
    e. Transfusion reactions records.
    f. Record of bacteriological studies.
    g. Record of refrigeration temperatures.
    h. Record of blood inspection.
    i. Record of blood received from outside source.
    j. Laboratory work records.

To this should be added distribution records, in the event that the blood bank ships blood.

3. The length of time records should be kept varies in each State.

Suggested reading assignments for students

AABB, Technical Methods, pp. 57–58, 92.
PHS, *Biological Products*, pp. 19.

Study Questions

1. Is it a good idea to write test results on a slip of paper, then copy them neatly in the laboratory record? If not, why not?
2. What is the way to correct an error in the records when they are indelible?
3. Why are records of blood storage temperatures kept?
4. How long must we keep our blood bank records?

Unit VIII Routine Analysis

Suggested Time: 6 weeks; 240 hours.

Unit content

A. Introduction to Urinalysis.
B. Specific Gravity and pH of Urine.
C. Urinary Sugars.
D. Urinary Proteins.
E. Ketone Bodies.
F. Bilirubin, and Urobilinogen.
G. Microscopic Exam of Urinary Sediment: Part I, Introduction and Organized Sediment.
H. Microscopic Exam of Urinary Sediment: Part II, Unorganized Sediment, Miscellaneous Structures, Addis Count, and Review.
I. Sulkowitch Test for Qualitative Urine Calcium.
J. Phenosulphonphthalein Excretion Test (PSP).
K. Gastric Analysis.
L. Examination of Feces for Neutral Fat and Occult Blood.

Note: Samples of student work sheets for routine urinalysis, urine microscopies, Sulkowitch test, PSP test, gastric analysis, and occult blood are given at the end of this unit.

Bibliography

Davidsohn, I., and Wells, B. B., *Clinical Diagnosis by Laboratory Methods,* 13th ed., Philadelphia, W. B. Saunders, 1962.

Dorland, W. A., *The American Illustrated Medical Dictionary,* 23d ed., Philadelphia, W. B. Saunders, 1958.

Dukes, C. E., *Urine Examination and Clinical Interpretation,* London, Oxford University Press, 1939.

Hepler, Opal E., *Manual of Clinical Laboratory Methods,* 4th ed., Springfield, Ill., Chas. C. Thomas, 1958.

Kolmer, John A., *Clinical Diagnosis by Laboratory Examinations,* 3d ed., New York, Appleton-Century-Crofts, Inc., 1961.

Lippman, R. W., *Urine and Urinary Sediment,* Springfield, Ill., Chas. C. Thomas, 1952.

Page, Lot B., and Culver, Perry J., *Syllabus of Laboratory Examinations in Clinical Diagnosis,* Cambridge, Mass., Harvard University Press, 1960.

Simmons, J. S., and Gentzkow, C. J., *Medical and Public Health Laboratory Methods,* Philadelphia, Lea and Febiger, 1955.

Watson, C. J., *Outlines of Internal Medicine,* 9th ed., Part V, Dubuque, Iowa, Wm. C. Brown Co., 1958.

Booklets

Medical Laboratory Assistant Course, *L.A. 7—Urinalysis Manual,* Minneapolis, Minn., University of Minnesota, 1960.

Medical Laboratory Assistant Course, L.A. 7—*Urinalysis Laboratory Exercise Manual,* Minneapolis, Minn., University of Minnesota, 1961.

Medical Laboratory Assistant Course, *L.A. 8—Urinalysis Manual,* Minneapolis, Minn., University of Minnesota, 1959.

Urine as the Index of Health and Disease, Ames Pictoclinic, Vol. 5, Number 4, The Ames Co., Inc., Elkhart, Ind.

The Neglected Art of Urine Testing, Ames Pictoclinic, Vol. 6, Number 9, The Ames Co., Inc., Elkhart, Ind.

The Significance of Proteinuria, Ames Pictoclinic, Vol. 5, Number 9, The Ames Co., Inc., Elkhart, Ind.

Unit outline

A. Introduction to Urinalysis:
  1. Brief review of the kidney:
    a. Diagram of the gross anatomy of the kidney.
    b. Function of the kidney.
  2. Purpose and definition of urinalysis.
  3. Properties of urine in a normal individual:
    a. Color—amber or straw color in health.
    b. Reaction—normally has a slight acid reaction—pH 6 (range 4.8–7.5).
    c. Odor—normally has a peculiar distinctive odor.
    d. Taste—normally has a bitter saline (salty) taste.
    e. Urine volume—average quantity excreted in 24 hours in a man in good health is 1200–1600 ml. (approx. 3 pints).
    f. Specific gravity—1.015 to 1.025 in a normal individual.
  4. Composition of urine:
    a. Made up of 960 parts water and 40 parts solids.
    b. Composition of solid matter in normal urine.
      (1) Organic—urea (40–50% of solids excreted), uric acid, creatinine.
      (2) Inorganic—chlorides, phosphates, sulfates, ammonia.
    c. Abnormal substances found in urine in various conditions:
      (1) Acetone, albumin, bile, blood, pus, hemoglobin, glucose, fat.
      (2) Cystine, spermatozoa, epithelial cells, sulfonilamides, casts, bacteria, parasites.
  5. Routine urinalysis—usually consists of color, transparency, specific gravity, pH, protein, sugar (acetone and diacetic acid when indicated), and microscopic examination.
  6. Collection of urine specimens:
    a. Types of containers for collecting urine.
    b. Preservation of urine specimens:
      (1) Changes that occur in urine standing at room temperature for several hours.
      (2) Methods of preservation—refrigeration, toluol or thymol.
    c. Voided urine specimens.
    d. Catheterized urine specimens.
    e. Collection of urine for:
      (1) Routine urinalysis.
      (2) Qualitative urine tests.
      (3) Quantitative urine tests.
  7. Physical properties of urine:
    a. Urine volume:
      (1) Measurement and usage.
      (2) Terminology: polyuria, oliguria, anuria, nocturia, diuresis.
    b. Color:
      (1) Normal—yellow (due primarily to urochrome); varies from pale straw to a deep amber color.
      (2) Abnormal colors—pale, dark yellow, brown-red, yellow-brown, or beer brown, orange-red or orange-brown, red, dark brown or black.

c. Transparency:
  (1) Normal—clear when voided.
  (2) Abnormal—cloudiness may be due to mucin or mucous threads, amorphous phosphate crystals, bacteria, amorphous urate crystals, pus, blood, or fat.

d. Odor (due to volatile acids) and their causes:
  (1) Ammoniacal odor, (2) "Urinod," (3) Fruity odor, (4) Odor of mercaptans, (5) Odor in urinary tract infections.
  (1) Normal—white foam.
  (2) Abnormal—yellow (indicative of presence of bile pigments).

8. Urinalysis equipment:
  a. Usage and types.
  b. Care of equipment:
    (1) Cleanliness, (2) Proper cleaning procedure.

9. Personal cleanliness in the urinalysis laboratory.

Audiovisual Aids

The Role of Urine in Diagnosis, filmstrip, Parts 1 and 2, Audiovisual Seminars 100 and 101, Commission on Continuing Education, American Society of Clinical Pathologists, Chicago, Ill.

Work of the Kidneys, B. & W., sound, 11 min., Encyclopaedia Britannica Films, Wilmette, Ill.

Introduction to Laboratory Method

1. Equipment:
  a. Issued to each student: urinometer and cylinder, universal pH paper and color scale, eye droppers and bottle, test tubes (25 x 150 mm. and 16 x 150 mm.), centrifuge tubes, test tube rack, glass marking pencil.
  b. To use for exercise on physical properties of urine: test tubes and rubber stoppers.
2. Specimens used:
  a. Demonstration urine specimens for color (8 urines), transparency (6 urines), odor (3 urines), and foam (3 urines).
  b. Unknown urine specimens—four urine specimens to be tested for color, transparency, odor, and foam.

Laboratory Exercises

1. Review of urinalysis equipment:
  a. Become familiar with the names, usage, and care of the equipment.
  b. Wash all urinalysis glassware by the proper cleaning procedure.
2. Physical properties of urine:
  a. Brief review of the physical properties of urine.
  b. Demonstration of the proper technique for doing color, transparency, odor, and foam on urine specimens—what to look for.
  c. Student exercise.
    (1) Examine the demonstration urine specimens for physical properties (8 for color, 6 for transparency, 3 for odor and 3 for foam).
      (a) Record observations by sight, smell, or shaking.
      (b) List the probable cause or causes for each.
    (2) Unknown urine specimens—Record the results on four unknown urine specimens for color, transparency, order, and foam.

Study Questions

1. Draw the kidney, ureter, bladder, and urethra.
2. Briefly list the steps used in washing urinalysis equipment.
3. Give the procedure for the collection of a 24 hour specimen.
4. Name three ways to preserve urine.
5. What care must be exercised in the use of preservatives?
6. Name five reasons why it is important to examine a urine specimen immediately after voiding.
7. Why is it important to record the physical properties on a urine specimen?
8. What color, transparency, odor, and foam would you expect in a *normal* urine?
9. Briefly describe how you would collect the urine for the following tests:
  a. Routine urinalysis.
  b. Qualitative urine tests.
  c. Quantitative urine tests.
10. Name the normal constituents of urine.

11. Name five colors seen in urine and their probable causes.
12. Name four substances which affect the odor of the urine and their probable causes.

B. Specific Gravity and pH of Urine:

1. Specify gravity:
   a. Definition of specific gravity.
   b. Purpose of doing specific gravity on urine.
   c. Determination of specific gravity by use of a urinometer.
      (1) Means of testing a new urinometer.
      (2) Calibration of urinometer with distilled water and potassium sulfate solutions.
   d. Specific gravity corrections.
      (1) Temperature correction for precise work—0.001 should be added to the reading for each 3°C. *above* standard temperature and 0.001 should be subtracted for each 3°C. *below* standard temperature.
      (2) Correction for protein and sugar in the urine:
         (a) When urine contains 1 percent (1 gm. protein or sugar per 100 ml. of urine) protein or sugar, 0.003 must be subtracted from the specific gravity.
         (b) Quantitative tests for urinary sugar and protein are run to determine the exact amount present.
   e. Specific gravity of urine:
      (1) Normal value is from 1.015 to 1.025.
      (2) Pathological range is from 1.001–1.060.
      (3) Generally, in disease and in health, the specific gravity of urine varies inversely with the volume of the urine with the following exceptions: diabetes mellitus and terminal nephritis.
   f. Procedure for determining the specific gravity of urine:
      (1) Check cleanliness of urinometer cylinder.
      (2) Check urinometer daily for accuracy with standard solution and make correction if necessary.
      (3) Using well-mixed urine specimen, read the urinometer.
      (4) Requirements for reading the urinometer: clean urinometer, no bubbles around urinometer, urinometer should be floating freely, read on flat surface, read at bottom of meniscus, make urinometer correction if necessary.
2. pH:
   a. Definition of pH.
   b. pH values in normal urine.
      (1) Normal urine pH is 6.0 (range of 4.8–7.5).
      (2) Freshly voided urine is almost always acid in reaction.
   c. Determination of urinary pH.
      (1) Litmus paper.
      (2) Nitrazine paper (universal pH paper) pH range of acid, pH 4 to pH 7, alkaline.
      (3) Combistix (Ames) pH range 4.5–9.0.
   d. Significance of an alkaline reaction in urine.
      (1) Freshly voided urine.
      (2) Decomposition upon standing at room temperature for several hours due to the breakdown of urea by bacteria to form ammonia.
   e. Significance of increase in acidity.
      (1) Drugs (3) Fever
      (2) Acidosis (4) Excess protein
   f. Precautions.
      (1) Freshly voided urine specimen.
      (2) Caution in using preservatives.

Introduction to Laboratory Method

1. Equipment.
   a. Urinometer and cylinder.
   b. pH paper and color scale.
   c. Stirring rod.
   d. Marking pencil.
2. Specimens used.
   a. Urine specimens with known specific gravity and pH.
   b. Unknown urine specimens—4 urine specimens to be tested for specific gravity and pH.

Laboratory Exercises

1. Review of technique for doing specific gravity and pH on urine.
2. Demonstrations.
   a. Clean and dirty urinometers (note meniscus).
   b. Urine specimens with known specific gravities and pH.
3. Student exercise.
   a. Check urinometer for accuracy.
   b. Observe demonstration specimens for specific gravity and pH.
   c. Perform the following tests on four urine specimens: color, transparency, odor, foam, specific gravity, and pH.
   d. Record the results on a chart.
4. Acceptable student performance.
   a. Specific gravity of + 0.002 from correct result.
   b. pH should be ± 0.5.

Study Questions

1. Define: a. specific gravity. b. pH.
2. How would you obtain the specific gravity with a small amount of urine?
3. Give the technique for determining specific gravity.
4. What is the specific gravity of normal urine?
5. What is the pH of normal urine?
   a. Range: ________
   b. Average: ________
6. What changes occur in the pH after urine has been standing at room temperature for several hours? Why?
7. Give the technique for determining pH.
8. Name two substances in urine which will increase the specific gravity.
9. Of what significance is a high specific gravity?
10. Of what significance is a low specific gravity?
11. How would you make a temperature correction on the specific gravity of urine?

C. Urinary Sugars.

1. General considerations.
   a. Utilization of carbohydrates by the body.
   b. Storage of carbohydrates.
   c. Excretion of carbohydrates.
2. Glucose (glucosuria or glycosuria).
   a. Definition.
   b. Causes.
   c. Tests for detecting glucose in urine—depend largely upon ability to reduce certain substances (reducing ability due to aldehyde nature).
      (1) Other reducing substances found in urine—uric acid, creatine, fructose, lactose, pentose, homogentisic acid, glucuronic acid, chloroform, formaldehyde.
      (2) When substance with reducing ability is detected in the urine and there is any doubt on clinical grounds that substance is glucose, a fermentation test with yeast should be carried out.
3. Screening tests for glucose (to tell whether reducing substance is present or not):
   a. "Galatest"—(commercial test—Denver Chemical Manufacturing Co.)
      (1) Principle: bismuth oxide is reduced by urine sugar to a finely divided black metallic bismuth (grey or black) color, indicates a positive test.
      (2) Contents of powder.
      (3) Precautions.
      (4) Procedure.
      (5) Interpretation of results.
   b. "Tes-tape" and "Clinistix"—enzyme tests using glucose oxidase and are specific for glucose (Tes-tape—Lilly Co.; Clinistix—Ames Co.)
      (1) Principle.
      (2) Contents of paper strip.
      (3) Nature of chemical reaction.
      (4) Procedure.
      (5) Interpretation of results.
4. Qualitative tests for urine sugar (to give rough estimate of amount present).
   a. Benedict's qualitative test—reduction of copper sulfate.
      (1) Principle: ability of glucose to reduce cupric hydroxide to cuprous oxide.
      (2) Reagent used.
      (3) Procedure for doing test.

(4) Interpretation of the test—grading, amount of precipitate, color changes in supernatant and in mixed solution, approximate percent glucose present.
b. "Clinitest" (commercial tables—Ames Company).
(1) Principle.
(2) Contents of tablet.
(3) Precautions on using tablets.
(4) Procedure.
(5) Interpretation of results.
c. Fermentation test.
(1) Principle.
(2) Reagents.
(3) Procedure.
(4) Results of test.

5. Quantitative tests for urine sugar.
a. General considerations.
(1) Use and procedure for dilution.
(2) Use and procedure for removal of protein.
b. Benedict's quantitative method.
c. Somogyi method.
d. Fermentation test.

Introduction to Laboratory Method

1. Introduction to urine sugars using primarily screening and qualitative tests.

2. Equipment.
a. 16 x 150 mm. test tubes.
b. Eye droppers and bottles.
c. Stirring rod.
d. Marking pencil.
e. Boiling water bath } with racks.
Cold water bath }

3. Reagents.
a. Benedict's qualitative reagent.
b. Clinitest tablets and color charts.
c. Tes-tape paper and color chart.
d. Clinistix strips.
e. Galatest powder.

4. Specimens used.
a. Normal and abnormal urine specimens.
b. Four unknown urine specimens for urine sugars.

5. Methods used: Benedict's, Clinitest, Galatest, Clinistix, and Tes-tape.

Laboratory Exercises

1. Explain and review principle and technique for doing tests for urine sugars.

2. Demonstrate Benedict's urine sugars and normal and abnormal urine specimens with Clinitest, Galatest, Clinistix, and Tes-tape.

3. Student exercise.
a. Look at demonstration specimens of Benedict's urine sugars and record results as to supernatant color, amount of precipitate, color of mixed solution, and final result.
b. Look at demonstration of commercial tests using normal and abnormal urine.
c. Using Benedict's qualitative test, determine urine sugars on four unknown specimens.
(1) Follow directions given in lecture.
(2) Record results as to supernatant color, amount of precipitate, color or mixed solution, and final result.
d. Do commercial tests (Clinitest, Clinistix, Tes-tape, and Galatest) for urine sugars on two of the four specimens provided.

Name of commercial test	Detects	Major component of reagent, strip tablet, or powder	Negative result (color)	Positive result (color)	UNKNOWN SPECIMENS
Clinitest					
Clinistix					
Galatest					
Tes-tape					

(1) Follow directions given in lecture.
(2) Record results:

4. Acceptable student performance is ability to grade the results accurately.

Study Questions

1. Draw in chart from the tests for reducing sugars in urine and include the following:
   a. Name of test.
   b. Major components of reagent used in the test.
   c. Brief description of the test method.
   d. Description of the interpretation of the results of the test.
2. What is the most common sugar present in the urine? List other sugars that may be present.
3. What is each of the following:
   a. A screening test?
   b. A qualitative test?
   c. A quantitative test?
4. Name the following:
   a. 3 screening tests for urine sugars.
   b. 2 qualitative tests for urine sugars.
5. How are the results for the following recorded:
   a. Screening tests for urine sugars.
   b. Qualitative tests for urine sugars.
   c. Quantitative tests for urine sugars.
6. Name 2 tests which are specific for glucose. What do the other tests detect?
7. Name 3 conditions in which you would expect to find sugar in the urine.
8. How would you differentiate whether a reduction of Benedict's solution is due to glucose or lactose?
9. Name two tests for urine sugars which you prefer and for which you would have the most confidence in your results.
10. Name 6 reducing substances found in the urine.
11. Define:
    a. renal glucosuria. b. renal threshold.

D. Urinary Proteins.

1. Introduction.
   a. Derivation of urinary proteins.
   b. Constituents of urinary protein—serum albumin, serum globulin, mucin, proteoses.
   c. Causes of proteinuria.
2. Testing the urine for protein.
   a. Requirements of the specimen.
      (1) Clear urine by filtration or by centrifugation.
      (2) Removal of mucin by acidifying with acetic acid and filtering.
      (3) Urine voided early in evening or a few hours after a meal—most likely to contain protein.
      (4) Dilution of extremely concentrated urine.
   b. Causes of false positive tests for protein.
3. Qualitative tests for determining protein in the urine:
   a. Principle:
      (1) Means of precipitation of protein.
      (2) Sources of error.
   b. Ring or contact tests:
      (1) Robert's test.
      (2) Heller's test.
   c. Precipitation tests (heat tests):
      (1) Principle.
      (2) Exton's sulfosalicylic acid.
      (3) Heat and nitric acid test.
      (4) Salt-acetic test (Purdy's):
         (a) Principle of test.
         (b) Reagent.
         (c) Procedure.
         (d) Interpretation of results.
      (5) "Bumintest" (tablet—Ames Company):
         (a) Principle of test.
         (b) Contents of the tablet.
         (c) Precautions—stability of tablet, urine turbidity, and interfering substances.
         (d) Procedure.
         (e) Interpretation of results.
   d. Colorimetric tests:
      (1) "Albutest" (tablet—Ames Company):
         (a) Principle of the test.
         (b) Contents of the tablet.
         (c) Procedure.
         (d) Interpretation of results.

- (2) "Albustix" (reagent strips—Ames Company):
    - (a) Principle of test.
    - (b) Contents of the strip.
    - (c) Precautions.
    - (d) Procedure.
    - (e) Interpretation of results.
- (3) "Uristix" (reagent—Ames Company):
    - (a) Colorimetric combination test for protein and glucose in urine.
    - (b) Principle of test.
    - (c) Contents of strip—protein testing area and glucose testing area.
    - (d) Precautions.
    - (e) Procedure.
    - (f) Interpretation of results.
- (4) "Combistix" (reagent strip—Ames Company):
    - (a) A "dip and read" test for the determination of proteinuria, glycosuria, and urine pH.
    - (b) Principle of test.
    - (c) Contents of strip.
    - (d) Precautions.
    - (e) Procedure.
    - (f) Interpretation of results.

4. Quantitative tests for determining protein in the urine:
    - a. Principle:
        - (1) Types of specimens required.
        - (2) Value of quantitative determinations in nephrosis and nephritis.
    - b. Esbach method:
        - (1) Principle.
        - (2) Reagent.
        - (3) Procedure.
        - (4) Esbach tube.
        - (5) Method of reporting.
    - c. Biuret method:
        - (1) Principle.
        - (2) Reagents.
        - (3) Procedure.
        - (4) Method of reporting.
5. Bence-Jones protein:
    - a. Introduction:
        - (1) Type of protein.
        - (2) Detection of protein.
        - (3) Characteristics of protein.
    - b. Principle of test.
    - c. Reagents.
    - d. Procedure.
    - e. Interpretation of results.
    - f. Paper electrophoresis—all urines positive for Bence-Jones protein should be checked using electrophoresis pattern.
6. Mucin:
    - a. Introduction.
    - b. Method for detection.
    - c. Causes for mucin in the urine.

Introduction to Laboratory Method

1. Introduction to urine proteins using qualitative tests (both precipitation and colorimetric tests).
2. Equipment:
    - a. Graduated centrifuge tubes—16 x 150 mm.
    - b. Graduated cylinder / Erlenmeyer flash } for making Bumintest reagent.
    - c. Centrifuges.
    - d. Boiling water bath / Cold water bath } with racks.
    - e. Esbach tubes for demonstration.
    - f. Stirring rod.
    - g. Glass marking pencil.
    - h. Small squares of paper.
3. Reagents:
    - a. Salt-acetic acid reagent.
    - b. Esbach reagent.
    - c. Bumintest tablets.
    - d. Albutest tablets.
    - e. Albustix strips.
    - f. Combistix strips.
    - g. Uristix strips.
4. Specimens used.
    - a. Normal and abnormal urine specimens.
    - b. Four unknown urine specimens.
5. Methods used: Salt-acetic acid, Bumintest, Albutest, Albustix, Combistix, and Uristix.

Laboratory Exercises

1. Explain and review principle and techniques.
2. Demonstrate urine proteins using the salt-acetic acid method, Bumintest, Albutest, Albustix, Combistix, and Uristix. Demonstrate Esbach method.
3. Student exercise:

- a. Observe demonstration specimens of urine proteins (salt-acetic acid method), and record results as to amount of precipitate and result.
- b. Observe demonstration of commercial tests using normal and abnormal urine.
- c. Observe demonstrations of quantitative urine proteins (Esbach method).
- d. Using the salt-acetic acid test and Bumintest, determine proteins on the four unknown urine specimens. Record the results as to amount of precipitate and grade.
- e. Using Albutest, Albustix, Combistix, and Uristix, for urine proteins on two of the four specimens provided. Record the results in chart form similar to the one used for results with commercial tests for urine sugars and give the name of the commercial test (Bumintest, Albutest, Albustix, Combistix, Uristix), major component or reagent, tablet, or strip, negative and positive results (turbidity or color), and unknown specimen results.

Study Questions

1. Draw in chart form the tests for protein in the urine and include the following:
   - a. Name of test (salt-acetic acid, Ex-Ton's, Purdy's, Robert's, Bumintest, Albustix, Albutest, Combistix, Uristix).
   - b. Major components or reagent used in the test.
   - c. Brief description of the test method.
   - d. Description of the interpretation of the results of the test.
2. Why should the urine for protein tests be clear and acid?
3. List four possible causes for a turbid or cloudy urine.
4. What are the chief constituents of urinary protein?
5. Give the procedure for Esbach's quantitative test for protein and draw an Esbach tube.
6. Give the procedure for identification of Bence-Jones protein.
7. In what condition is Bence-Jones protein present in urine?
8. What is the significance of the presence of protein in the urine?
9. When are quantitative determinations of the daily excretion of protein necessary?
10. Name two diseases in which the urine protein is elevated.

E. Ketone Bodies:

1. Introduction:
   - a. The three ketone bodies: Acetone, diacetic acid, and beta hydroxybutyric acid.
   - b. Clinical significance of ketone bodies in urine.
   - c. Origin of the ketone bodies.
   - d. Chemical nature of the ketone bodies.
   - e. Definitions: ketonuria, acidosis, ketosis.
2. Acetone:
   - a. Definition.
   - b. Occurrence:
     - (1) Mild acidosis.
     - (2) Severe acidosis.
     - (3) In children.
   - c. Methods for determining acetone:
     - (1) Rothera's test (sodium nitroprusside):
       - (a) Sensitivity of test.
       - (b) Principle of test.
       - (c) Reagents used.
       - (d) Procedure.
       - (e) Interpretation of results.
     - (2) Acetest (commercial tablet—Ames Company):
       - (a) Principle of test.
       - (b) Contents of the tablet.
       - (c) Precautions.
       - (d) Procedure.
       - (e) Interpretation of results.
3. Diacetic acid (aceto-acetic acid):
   - a. Definition.
   - b. Occurrence.
   - c. Methods for determining diacetic acid:
     - (1) Gerhardt's $FeCl_3$ test:
       - (a) Principle of test.
       - (b) Reagent used.
       - (c) Procedure.
       - (d) Interpretation of results; how to check for false positive results

and compounds giving false positive results.
      - (2) "Ketostix" (commercial dipstick test—Ames Company):
        - (a) Principle of test.
        - (b) Sensitivity of test.
        - (c) Specificity of test.
        - (d) Interpretation of results.
- 4. Beta-hydroxybutyric acid:
  - a. Definition.
  - b. Occurrence.
  - c. Hart's test described (clinical significance; why not done routinely):
    - (1) Principle.
    - (2) Reagent used.
    - (3) Sensitivity of test.
    - (4) Specificity of test.
    - (5) Interpretation of results.

Introduction to Laboratory Method

- 1. Equipment:
  - a. 15 x 150 mm. test tubes.
  - b. Boiling water bath.
- 2. Reagents used:
  - a. Rothera's reagent.
  - b. Concentrated ammonium hydroxide.
  - c. 10 percent ferric chloride.
  - d. "Acetest" tablets.
  - e. "Ketostix" strips.
- 3. Specimens used:
  - a. Four unknown urine specimens for acetone and diacetic acid.
  - b. Demonstration specimens of negative and positive results.
- 4. Methods used:
  - a. Acetone—Rothera's test and "Acetest."
  - b. Diacetic acid—Gerhardt's test.

Laboratory Exercises

- 1. Review principle and demonstrate technique for performing the tests for acetone and diacetic acid.
- 2. Demonstrate specimens of negative and positive results for acetone and diacetic acid.
- 3. Student exercises:
  - a. Observe the demonstration specimens for acetone and diacetic acid.
  - b. Perform the Rothera's test for acetone on the four unknown urine specimens.
  - c. Perform the Gerhardt's test for diacetic acid on the four unknown urine specimens.
  - d. Perform the commercial test "Acetest" on two of the four specimens provided.
  - e. Record the results in chart form for all specimens and test.

Study Questions

1. Under what conditions are tests for ketone bodies performed?
2. Name three ketone bodies which are found in the urine.
3. Which of the three ketone bodies exists in the smallest quantities in the urine? Which is said to be most toxic?
4. Of what clinical significance is the beta-hydroxybutyric acid test, and why is it not done routinely?
5. Name four pathological conditions in which acetone is present in the urine.

F. Bilirubin and Urobilinogen.

- 1. Bilirubin:
  - a. Introduction:
    - (1) Origin in body.
    - (2) Mechanism of presence of bilirubin in urine.
    - (3) Significance of bilirubin in urine.
    - (4) Constituents and derivatives of bile which appear in the urine:
      - (a) Bile pigments—bilirubin, biliverdin.
      - (b) Bile acids.
      - (c) Urobilin.
      - (d) Urobilinogen.
    - (5) Physical properties of bilirubin.
    - (6) Stability of bilirubin in urine.
  - b. Methods for determining bilirubin in the urine:
    - (1) Foam test:
      - (a) Procedure.
      - (b) Interpretation of results.
    - (2) Gmelin's test:
      - (a) Procedure.
      - (b) Sensitivity.
      - (c) Interpretation of results.
    - (3) Harrison's spot test:
      - (a) Principle.
      - (b) Reagents used.
      - (c) Procedure.

        - (d) Interpretation of results.
      - (4) "Ictotest" (commercial tablet—Ames Company):
        - (a) Principle.
        - (b) Contents of tablet.
        - (c) Precautions.
        - (d) Procedure.
        - (e) Interpretation of test.

2. Urobilinogen:
   - a. Introduction:
     - (1) Origin in body.
     - (2) Mechanism of presence of urobilinogen in urine.
     - (3) Significance of urobilinogen in urine.
     - (4) Physical properties of urobilinogen.
     - (5) Stability of urobilinogen in urine.
   - b. Methods for determining urobilinogen in the urine:
     - (1) Ehrlich's aldehyde test:
       - (a) Principle.
       - (b) Reagents.
       - (c) Procedure.
       - (d) Interpretation of results:
         - Differentiation between urobilinogen and porphobilinogen and indol.
         - Compounds which may give false positive reactions with Ehrlich's aldehyde reagent.
   - c. Determination of urobilin in urine:
     - (1) Schlesinger's test.
     - (2) Interpretation of results.
     - (3) Significance of abnormal results.

Introduction to Laboratory Method

1. Equipment:
   - a. 16 x 150 mm. test tubes.
   - b. Eye dropper and bottle.
   - c. Rubber stoppers or #4 corks.
2. Reagents used:
   - a. Saturated barium chloride strips.
   - b. Fouchet's reagent.
   - c. Ehrlich's reagent.
   - d. Saturated sodium acetate.
   - e. Chloroform.
   - f. "Ictotest" tablets and filter papers.
3. Specimens used:
   - a. Four unknown urine specimens for bilirubin and urobilinogen.
   - b. Demonstration specimens of negative and positive results for bilirubin and urobilinogen.
4. Methods used:
   - a. Bilirubin—Harrison's test and "Ictotest."
   - b. Urobilinogen—Ehrlich's test.

Laboratory Exercises

1. Review principle and demonstrate technique for tests for bilirubin and urobilinogen in urine.
2. Demonstrate specimens of negative, positive, and false positive results for bilirubin and urobilinogen.
3. Student exercises:
   - a. Observe the demonstration specimens for bilirubin and urobilinogen.
   - b. Perform the Harrison's test for bilirubin on the four unknown urine specimens.
   - c. Perform the Ehrlich's test for urobilinogen on the four unknown urine specimens.
   - d. Perform the commercial test "Ictotest" on two of the four specimens provided.
   - e. Record the results in chart form for all specimens and tests.

Study Questions

1. In what pathological conditions is the urine examined for bilirubin? For urobilinogen?
2. Bilirubin gives a (n) ____________ color to the urine and is detetced in the laboratory by means of (name) test. A positive test is indicated by a (n) ____________ color which is due to ____________, a (n) product of bilirubin. The reagent used to detect bilirubin in urine is ____________ and is composed of ____________ and ____________.
3. Upon what basic principle do tests for bile depend?
4. ____________ and ____________ give a positive reaction with Ehrlich's reagent. Explain how you can distinguish between the two.
5. Give two reasons for an increase in the amount of urobilinogen in the urine.

F. Microscopic Examination of Urinary Sediment—Part I: Introduction and Organized Sediment:

1. Introduction:
   a. Definition of urinary sediment.
   b. Requirements of specimens for microscopic examination of urinary sediment:
      (1) Clean specimen.
      (2) Fresh first morning specimen.
      (3) Well-concentrated specimen.
      (4) Well-mixed specimen.
   c. Preparation of specimen for microscopic examination:
      (1) Centrifuge 10 ML of well-mixed specimen at 3000 R.P.M. for five minutes.
      (2) Decant quickly.
      (3) Mix remaining liquid with sediment.
      (4) Place drops on slide, cover with coverslip.
   d. Direct examination of the sediment:
      (1) Scanning.
      (2) Enumeration of the sediment:
         (a) Average number of rbc/high power field.
         (b) Average number of wbc/high power field (note presence and size of clumps).
         (c) Average and types of casts per low power field.
         (d) Unorganized sediment (list kind and whether few, moderate, or many/high power field).
         (e) Note presence of bacteria, yeast, epithelial cells, etc.
   e. Types of urinary sediment:
      (1) Organized sediment (greatest importance).
         (a) Definition.
         (b) Contents of organized sediment: red blood cells, white blood cells, epithelial cells, casts, bacteria, parasites, yeast, and fungi.
      (2) Unorganized sediment:
         (a) Definition.
         (b) Contents of unorganized sediment: crystals and amorphous material.
2. Organized sediment:
   a. Red blood cells (rbc)
      (1) Significance and source.
      (2) Description:
         (a) Normal.
         (b) Changes in appearance resulting from varying osmolarity.
      (3) Substances most easily confused with red blood cells and how to differentiate them from red blood cells.
      (4) Reasons for occurrence in urine.
      (5) Manner of reporting results.
   b. White blood cells (wbc):
      (1) Significance and source.
      (2) Description.
      (3) Substances most easily confused with white blood cells and how to differentiate them from white blood cells.
      (4) Reasons for occurrence in urine.
      (5) Manner of reporting results.
   c. Casts:
      (1) Definition.
      (2) Sites of formation.
      (3) Significance of casts in the urine.
      (4) Description.
      (5) Substances most easily confused with casts and how to differentiate them from casts.
      (6) Manner of reporting results.
      (7) Watson's classification of casts as found in Watson's *Outlines of Internal Medicine*, Part V.
      (8) Classification and formation of casts as found in Lippman's *Urine and Urinary Sediment:*
         (a) Hyaline casts.
         (b) Granular casts:
            - Finely granular casts.
            - Coarsely granular casts.
            - Cellular casts.
         (c) Waxy casts.
         (d) Fatty casts.
         (e) Broad casts.

Introduction to Laboratory Method

1. Review of technique in using microscope and in enumerating and looking for red blood cells, white blood cells, and casts.

2. Equipment:
   - a. Microscope and lamp.
   - b. Applicator sticks.
   - c. Glass slides.
   - d. Cover glasses.
3. Reagents used—glacial acetic acid.
4. Specimens used:
   - a. Demonstration specimens of red blood cells, white blood cells, and different types of casts.
   - b. Four *known* specimens with red blood cells and white blood cells.
   - c. Four *known* specimens with different kinds of casts.
   - d. Four *unknown* specimens with red blood cells and white blood cells.
   - e. Four *unknown* specimens with different kinds of casts.

Laboratory Exercises

1. Review the procedure for obtaining sediment to be examined:
   - a. Type of specimen.
   - b. Centrifuging.
   - c. Transfer of sediment to slide for examination.
   - d. Examination of sediment with the microscope.
   - e. Proper grading of results found.
2. Demonstrations:
   - a. Red blood cells (intact, crenated, and shadow).
   - b. White blood cells (clumps and single).
   - c. Various kinds of casts (show both under low and high power objectives of microscope).
3. Student work sheet for microscopic examination of the urine.
4. Student exercise:
   - a. Observe the demonstration specimens of red blood cells, white blood cells, and casts before beginning on your own.
   - b. Examine the four *known* urine specimens for red blood cells and white blood cells; grade and report them on the Student Work Sheet for Urine Microscopics.
   - c. Examine the four *known* urine specimens for casts; grade and report on Work Sheet.
   - d. When thoroughly familiar with appearance and examination of *known* specimens, examine the four *unknown* urine specimens for red blood cells, and white blood cells; grade and report.
   - e. Examine the four *unknown* urine specimens for casts; grade and report.
5. Acceptable student performance:
   - a. Ability to find red blood cells, white blood cells, and casts in urine.
   - b. Ability to grade red blood cells, white blood cells, and casts in urine.
   - c. Ability to identify the various kinds of casts found in urine sediment.

Study Questions

1. What is included in (a) organized sediment and (b) unorganized sediment?
2. Which structures are (a) *graded* under low power, (b) *graded* under high power?
3. What is the most satisfactory method of urine collection when a microscopic examination is requested?
4. Name four changes which might occur in the results of a microscopic examination if the urine has been sitting at room temperature for several hours before examined.
5. Name three conditions which are frequently associated with hematuria.
6. Name the following:
   - (a) Microscopic structures which might be mistaken for white blood cells under the microscope; state briefly how to differentiate from white blood cells.
   - (b) Three structures which might be confused with red blood cells; tell how you would differentiate them from red blood cells.
   - (c) Three microscopic structures which might be mistaken for casts; state how to differentiate them from casts.
7. Give four precautions necessary in mounting a drop of the urinary sediment on the glass slide.

8. The size of (a) a red blood cell is .... microns; (b) a white blood cell is ....micra.

9. What three forms of red blood cells may be found in the urine?

10. Name three of the most common errors which result in failure to find important structures in the urine by microscopic examination.

11. Briefly describe the following: (a) The appearance of a red blood cell in the urine; (b) The appearance of a white blood cell in the urine.

12. Complete the following: (a) The presence of red blood cells in the urine is called .... (b) The presence of white blood cells in the urine is called ....

13. How can you distinguish between hemoglobinuria and hematuria: (a) Microscopically; (b) Macroscopically.

14. (a) How are casts formed?
    (b) What are casts?
    (c) Which is the most common type of cast?
    (d) Name 4 kinds of casts and give their distinquishing characteristics.

H. Microscopic Exam of Urinary Sediment—Part II: Unorganized Sediment, Miscellaneous Structures, Addis Counts.

1. Unorganized sediment:
   a. Introduction:
      (1) Significance.
      (2) How to report results.
   b. Normal crystals of *acid* urine—description, significance, and diagram of each type of crystal:
      (1) Amorphous urates (sodium and potassium acid urate).
      (2) Uric acid crystals.
      (3) Calcium oxalate.
   c. Normal crystals of *alkaline* urine—description, significance, and diagram of each type of crystal:
      (1) Amorphous phosphates (tricalcium phosphate, magnesium phosphate, and calcium carbonate).
      (2) Triple phosphate (ammonium magnesium phosphate).
      (3) Ammonium biurate crystals.
      (4) Calcium phosphate.
      (5) Magnesium phosphate (rare).
      (6) Calcium carbonate.
   d. Abnormal crystals:
      (1) Description, significance, and diagram of each type of crystal.
      (2) Comparison with normal crystal with which it might be easily confused.
      (3) Abnormal crystals are not reported on microscopic evidence alone, but must have confirmatory chemical tests:
         (a) Cystine crystals.
         (b) Leucine and tyrosine crystals.
         (c) Cholesterol crystals.
         (d) Hippuric acid.
         (e) Sulfonamide crystals:
            - Sulfadiazin—free and acetylated forms.
            - Sulfapyridine.
            - Sulfathiazole.

2. Miscellaneous structures occurring in the urine:
   a. Epithelial cells:
      (1) Appearance.
      (2) Relation of site of origin to the size and shape.
      (3) Types of epithelial cells seen in urinary sediment.
   b. Urinary calculi.
   c. Bacteria and parasites:
      (1) Appearance and means of identification.
      (2) Significance of bacteria in urine.
   d. Parasites—Trichomonas vaginalis (or hominis).
   e. Yeast cells.
   f. Spermatozoa.
   g. Mold-fungi.
   h. Fibrin threads:
      (1) Appearance.
      (2) Occurrence with blood casts.
   i. Starch granules.
   j. Fat globules.
   k. Artifacts in the urinary sediment:
      (1) Fibers of cotton or wool.
      (2) Bubbles or air.
      (3) Dirt and scratches on slide.
      (4) Talcum powder.

3. The Addis sediment count:
   - a. Introduction:
     - (1) Important consideration in collection and examination of specimen.
     - (2) Procedure.
     - (3) Calculations.
     - (4) Results.
     - (5) Normal values for adults:
       - (a) Erythrocytes: 0 - 500,000/12 hours.
       - (b) Leukocytes: 0 - 1,000,000/12 hours.
       - (c) Epithelial cells: may increase to 2,000,000 - 5,000,000.
       - (d) Hyaline casts: 0 - 5,000/12 hours.
     - (6) Children normally may have more albumin and casts than adults; erythrocytes and leukocytes may be less than for adults.

4. Review of microscopic examination of urinary sediment:
   - a. Procedure for urine for urinary microscopic sediment examination:
     - (1) Centrifuge 10 ml. of well-mixed urine for 5 minutes at 3000 rpm.
     - (2) Decant 9 ml. of the supernatant and use for qualitative protein test.
     - (3) The remaining 1 ml. is mixed thoroughly with an applicator stick to insure equal distribution of the sediment.
     - (4) One or two drops of well-mixed sediment are transferred to a numbered glass slide with an applicator stick.
     - (5) A coverslip is placed carefully over the wet film.
     - (6) Precautions:
       - (a) Drop should be proper size so there will be no bubbles (not enough).
       - (b) Drop should be proper size so the coverslip will not float (too much).
   - b. Examination of wet film of urinary sediment under the microscope:
     - (1) Low power:
       - (a) Examine systematically.
       - (b) Look for casts and crystals:
         - ● Grade the number present per low power field after looking at a minimum of 10 lpf.
         - ● Identify the kind under high power.
     - (2) High power:
       - (a) Grade red blood cells and white blood cells according to the number present per high power field after looking at a minimum of 10 hpf.
       - (b) Identify the casts and crystals.
   - c. Grading of structures in urinary sediment.
     - (1) Importance of quantitative relationship of the sediment to the urine sample.
     - (2) Examine the sediment microscopically under reduced light and use the low-power objective first to gain crude estimation of the variety and quantity of elements present and to look for casts. The high-power objective is used for making finer distinctions and for enumerating the red blood cells and white blood cells.
     - (3) A minimum of 10 fields must be counted for an accurate determination of the number present.

Introduction to Laboratory Method

1. Review of technique for performing urinary sediment for microscopic examination for red blood cells, white blood cells, casts, normal and abnormal crystals, and miscellaneous structures.
2. Equipment:
   - a. Microscope and lamp.
   - b. Applicator sticks.
   - c. Glass slides.
   - d. Cover glasses.
3. Reagents used: glacial acetic acid.
4. Specimens used:
   - a. Demonstration of specimens of various kinds of normal crystals, abnormal crystals, and miscellaneous structures.

b. Four *known* specimens with normal crystals.
c. Four *known* specimens with abnormal crystals.
d. Four *known* specimens with miscellaneous structures.
e. Four *unknown* specimens with normal crystals.
f. Four *unknown* specimens with abnormal crystals.
g. Four *unknown* specimens with miscellaneous structures.

Laboratory Exercises

1. Microscopic examination of the urine for normal and abnormal crystals and miscellaneous structures:
   a. Review of procedure for examination and grading of normal and abnormal crystals and miscellaneous structures.
   b. Demonstrations of various kinds of normal and abnormal crystals and miscellaneous structures.
   c. Student exercises:
      (1) Examine the four *known* urine specimens for normal and abnormal crystals, and miscellaneous structures; grade and report.
      (2) When thoroughly familiar with the appearance and examination of the normal and abnormal crystals, and for miscellaneous structures grade, and report.
   d. Acceptable student performance: ability to find, grade, and identify normal and abnormal crystals and miscellaneous structures.
2. Urine Microscopics—Review:
   a. Specimen used—Four unknown urine sediments.
   b. Student exercises:
      (1) On the four unknown urine sediments, thoroughly examine the entire illumination:
         (a) Grade the casts, normal and abnormal crystals, and miscellaneous structures under low power.
         (b) Identify under high power.
      (2) Examine under high power:
         (a) Grade red blood cells, white blood cells.
         (b) Identify casts, normal and abnormal crystals, and miscellaneous structures.
      (3) Record the results.
   c. Acceptable student performance: ability to find, grade, and identify correctly all microscopic structures found in the urinary sediment.

Study Questions

1. Give the type of illumination, objective size, and total magnification used to grade the following microscopic structures:
   a. White blood cells.
   b. Hyaline casts.
   c. Red blood cells.
   d. Triple phosphates.
   e. Sulfonamides.
   f. Calcium oxalate.
   g. Tyrosine and leucine.
2. What would you expect to find microscopically in a specimen that visually appears (a) like "brick dust"? (b) cloudy red in appearance?
3. Name three structures which you might find microscopically if the urine specimen is turbid.
4. If the urine specimen contains protein, what microscopic structure should you particularly look for?
5. How are the following graded: (a) normal crystals (b) abnormal crystals?
6. Sulfonamide crystals are found in urine of what pH?
7. What is the clinical significance of finding sulfa crystals in the urine?
8. Draw the following structures from urinary sediments as they would appear magnified 430 diameters:
   a. Red blood cells (crenated, swollen, normal).
   b. Triple phosphate crystals.
   c. Ammonium biurate crystals.
9. Draw the following structures from urinary sediments as they would appear magnified 100 diameters:
   a. Squamous epithelial cells.
   b. Calcium oxalate crystals (2 forms).

c. Uric acid crystals (3 forms).
d. Granular casts.
e. Cylindroid.

10. a. Name three normal crystals found in acid urine.
b. Name three normal crystals found in alkaline urine.
c. Name four abnormal crystals found in urine.

11. What additional information is necessary in order to report the presence of abnormal crystals?

I. Sulkowitch Test for Qualitative Urine Calcium.

1. Introduction:
a. Excretion of calcium in the urine.
b. Important in study of bone disease.
c. Increase and decrease in urine calcium.
d. Normal values.

2. Introduction to laboratory method:
a. Principle of Sulkowitch test:
(1) Qualitative test for detecting the presence or absence of calcium in the urine.
(2) Ca^{++} + ammonium oxalate at pH 5 → Calcium oxalate (insoluble).
(3) Patient should be on a diet that is low in calcium and has a neutral ash for at least three days.
b. Equipment:
(1) Graduated centrifuge tubes.
(2) Rubber stoppers.
c. Reagents:
(1) Glacial acetic acid.
(2) Sulkowitch reagent (oxalic acid, ammonium oxalate, and glacial acetic acid).
d. Specimens used:
(1) Normal urine specimen to be used as a control.
(2) Four unknown urine specimens to be tested for urinary calcium.
e. Method:
(1) Collect urine for 24 hours, mix, measure, and record total volume.
(2) Centrifuge aliquot of urine if it is turbid or make a blank for it.
(3) Adjust aliquot to pH 5.
(4) Add Sulkowitch reagent, mix, allow to stand for 2 minutes.
(5) Set up a normal urine to be used as a control.
(6) If urine remains turbid after centrifuging, a blank must be set up to compensate for turbidity not due to calcium.
(7) Interpretation of results:
(a) The turbidity in the blank tube must be subtracted from the unknown tube before comparing to the control tube.
(b) The turbidity in the unknown tube is compared to that of the control urine.
(8) Results—graded according to turbidity present in unknowns when compared with control:
(a) Negative—no turbidity or precipitate.
(b) Less than the control.
(c) Same as the control.
(d) Greater than the control.
f. Quality control:
(1) Normal urine serves as comparison control.
(2) Abnormal urine to check on the reaction.
g. Clinical application:
(1) Results of "greater than the control" are obtained when there are abnormally large amounts of calcium present:
(a) Hyperparathyroidism.
(b) Tuberculosis.
(c) Diabetes.
(d) Febrile disease.
(2) Results of "less than the control" or "negative" are obtained when smaller than normal amounts of calcium are present:
(a) Hypoparathyroidism.
(b) Tetany.
(c) Rickets.
(d) Sprue, celiac disease.
h. Normal values: urine from normal subjects usually show faint turbidity.

Laboratory Exercises

1. Review and demonstration technique of Sulkowitch test:
a. Normal and abnormal controls.
b. Blank and unknown urines.

2. Student exercise:
    a. Do Sulkowitch tests on four unknown urine specimens using a normal urine as a control.
    b. Record results on Sulkowitch Test—Student Report Sheet.

Study Questions

1. What urinary constituent does the Sulkowitch test detect?
2. Briefly give the reaction which occurs in the Sulkowitch test. What could be the clinical significance of a high result? What could be the clinical significance of a low result?
3. Why is it important to adjust pH of urine before adding Sulkowitch reagent?
4. Why is it necessary to run a blank with cloudy urines?
5. For best results, the patient is put on a ______________ diet for ______ days before doing the Sulkowitch test.
6. Calcium in the urine is detected by ______ reagent which is made up of ______ and ______________ chemicals. It is used in the detection of disease. The principle of the method depends upon the precipitation of ______ by ______________ at pH ______________. The precipitate in the unknown urine is compared to a ______________ and graded as ______________, ______________, or ______________.

J. Phenosulphonphthalein Excretion Test:

1. Introduction:
2. Kidney function test.
    a. PSP—dye chiefly excreted by the kidney tubules.
    b. Excretion of dye dependent upon two factors:
        (1) The rate of renal blood flow
        (2) Tubular ability to excrete the dye
3. Introduction to laboratory method:
    a. Principle of laboratory test
        (1) The patient is injected with 6 mg. of PSP and the urine is collected at timed intervals.
        (2) Normally the amount of dye excreted is independent of urine volume.
    b. Equipment:
        (1) Stainless steel graduates.
        (2) 1000 ml. graduates (for measuring urine volume).
        (3) Stirring rod.
        (4) Hellige colorimeters.
        (5) Bausch & Lomb colorimeters.
    c. Reagents:
        (1) 20 percent sodium hydroxide.
        (2) Glacial acetic acid.
    d. Specimens used:
        (1) One set of four PSP urine specimens per student.
        (2) Standard PSP curve for the Bausch & Lomb colorimeter.
    e. Methods:
        (1) PSP test using Hellige colorimeter.
        (2) PSP test using Bausch & Lomb colorimeter (or other colorimeters).
    f. Sources of error:
        (1) Exact timing of specimens is necessary.
        (2) The test is unreliable unless the volume of each specimen is 50 ml. or more.
    g. Clinical application—In patients with decreased renal function, the diminution of excretion of dye is a rough index of the degree of renal damage.
    h. Normal values:
        (1) The maximum, minimum, and average normal values in percent of dye injected:
        (2) Recovery of 25 percent of dye injected is the lowest limit of normal for the 15 minute specimen.
        (3) Abnormal elimination of dye is re-

	15 min.	30 min.	60 min.	120 min.
Maximum	50 percent	24 percent	17 percent	10 percent
Minimum	28 percent	13 percent	9 percent	3 percent
Average	36 percent	18 percent	12 percent	7 percent

flected chiefly in the first 15 minute specimen, and so this is the most important specimen.

Laboratory Exercises

1. Review and demonstration of the technique of performing the PSP test, Hellige colorimeter, and Bausch and Lomb colorimeter.
2. Student exercise:
   a. Do PSP test on the four urine specimens from one patient, doing both methods using the Hellige colorimeter and the Bausch and Lomb colorimeter.
   b. Record results on Phenosulphonphthalein Excretion Test-Student Work Sheet.

Study Questions

1. The PSP test is a test to determine ________________. One milliliter containing ________________ milligrams of dye is injected intravenously.
2. Urine specimens are collected at ____________, ____________, ____________, and ________________ minute intervals after injection of the dye.
3. PSP dye is ________________ color in acid solution and ________________ color in alkaline solution; therefore ____________ is added to the urine after the ____________ has been measured and recorded.
4. The most important urine specimen is the ____________ specimen and the normal percent PSP dye excreted for this specimen is ____________ (give range of normals).
5. What was the difference between the results on the two colorimeters? Which colorimeter did you feel gave the most accurate reading and why?

K. Gastric Analysis:

1. Introduction to gastric analysis:
   a. Principle of gastric analysis.
   b. Method of performing the test on the patient.
   c. Stimulation of the gastric mucosa.
   d. Definition of degrees of acidity.
      (1) Term used to express the acidity found in the analysis of the gastric contents.
      (2) It represents the number of milliliters of 0.1 N NaOH required to neutralize 100 ml. of gastric juice.
      (3) Calculation of degrees of acidity.
   e. Normal values for a fasting specimen:

Volume of gastric juice	10–50 ml.
Free acid	5–20 degrees of acidity
Total acid	15–45 degrees of acidity

   f. Define
      (1) achlorhydria.
      (2) hyperchlorhydria.
      (3) hypochlorhydria.
2. Topfer's test:
   a. Principle of test—measurement of free acid.
   b. Reagent used.
   c. Procedure.
   d. False positive results.
   e. Normal values for free HCL: 5–20 degrees of acidity in fasting specimen.
3. Phenolphthalein test:
   a. Principle of test—measurement of "combined acid."
   b. Reagent used.
   c. Procedure.
   d. Normal values: total acid: 15–45 degrees acidity for fasting specimen. Combined acid = total acid − free acid = 10–15 degrees acidity for fasting specimen.
4. Boas test:
   a. Principle of test—specific for free HCL.
   b. When to use the test.
   c. Procedure.
5. Kelling's test for lactic acid:
   a. Principle.
   b. When to use test.
   c. Procedure.
   d. Reporting the presence or absence of lactic acid as: positive or negative.
6. Tubeless gastric analysis by use of the "Diagnex Blue" test:
   a. Principle of test—determine presence or absence of free HCL in the stomach by a tubeless technique.

- b. Procedure.
- c. Results of test.

Demonstrations

1. A short lecture by a physician on gastric analysis and demonstrate the technique of using the Levin duodenal tube on a patient or student.
2. Techniques for doing Topfer's test, phenolphthalein test, Boas test, and Kelling's test for lactic acid.
3. Specimens of various results obtained with Topfer's test, phenolphthalein test, Boas test, and Kelling's test for lactic acid.
4. "Diagnex Blue" test.

Introduction to Laboratory Method

1. Briefly review the principles of Topfer's test, the phenolphthalein test, Boas test, and Kelling's test for lactic acid.
2. Equipment:
   - a. Graduated cylinders—for measuring the total volume of the specimen and the aliquot of specimen used in the titration.
   - b. 50 ml. Erlenmeyer flasks.
   - c. Burette stand, rod, and base.
   - d. Stopcock grease and pipe cleaners.
   - e. Evaporating dishes.
   - f. Test tubes.
3. Reagents:
   - a. 0.1 N NaOH.
   - b. 0.5 percent Topfer's indicator.
   - c. 1 percent phenolphthalein.
   - d. Boas reagent.
   - e. 10 percent ferric chloride.
4. Specimens used: four gastric specimens (fasting, 20 minute, 40 minute, 60 minute).
5. Method:
   - a. Topfer's test for free acid, phenolphthalein test for combined acid.
   - b. Boas test for free acid in cases of low or no acidity, and Kelling's test for lactic acid.
6. Normal values:
   - a. Free acid (fasting specimen) = 5–20 degrees of acidity.
   - b. Combined acid (fasting specimen) = 10–15 degrees of acidity.
   - c. Total acid (fasting specimen) = 15–45 degrees of acidity.

Laboratory Exercise

1. Review the techniques of performing the Topfer's test, phenolphthalein test, Boas test, and Kelling's test for lactic acid.
2. Demonstrations:
   - a. Demonstrate technique and results of gastric specimens using Topfer's test, phenolphthalein test, Boas test, and Kelling's test for lactic acid.
   - b. Demonstrate the "Diagnex Blue" test.
3. Student exercise:
   - a. After watching the demonstrations, do gastric titrations on the four gastric specimens (fasting, 20 minute, 40 minute, and 60 minute) from patient, Mrs. X, who has a diagnosis of gastric ulcer.
   - b. Record results on Gastric Analysis—Student Work Sheet.

Study Questions

1. (a) What does the Boas test determine?
   (b) When do we do a Boas test?
2. Give the normal values for the following in a fasting specimen:
   - a. Free HCL.
   - b. Combined acid.
   - c. Total acid.
3. Do the results of the gastric specimens in the laboratory exercise (above) correlate with the diagnosis? Explain.
4. Name two of the most common gastric stimulants.
5. What is the purpose of doing an analysis on gastric contents?
6. If 4.1 ml. of O.1 N NaOH were used to neutralize 10 ml. of gastric juice, what would the degrees of acidity be?
7. Examine the results below for four patient results:
   - a. Which results are satisfactory?
   - b. Which results would you question?
8. Define:
   - a. Degrees of acidity.
   - b. Achlorhydria.
   - c. Hyperchlorhydria.
   - d. Hypochlorhydria.

	Patient X	Patient Y	Patient Z	Patient A
Free acid (fasting spec.).	0°	15°	20°	40°
Total acid (fasting spec.).	10°	45°	18°	60°
Lactic acid	neg	neg	neg	pos
Boas test	neg	neg	pos	neg

9. What is the advantage of doing a "Diagnex Blue" test?
10. Give the principle and procedure of the "Diagnex Blue" test.

L. Examination of the Feces for Neutral Fat and Occult Blood:

1. Neutral fat:
   - a. Introduction:
     - (1) Occurrence of fats: neutral fats, fatty acids, and soaps.
     - (2) Appearance of neutral fats.
     - (3) Diseases in which fats occur in the feces.
   - b. Procedure:
     - (1) Neutral fat is strained by mixing a small amount of feces with a saturated solution of Sudan III in 70 percent alcohol.
     - (2) The dye is dissolved by the fat globules, staining them an orange red.
     - (3) Fats are always present in feces in small amounts; therefore, their demonstration by staining methods must be interpreted cautiously.
     - (4) Chemical quantitation of total fat and expression in terms of dry weight of feces with the patient on a standard diet is a better indicator of the state of fat absorption but requires more elaborate laboratory facilities.
2. Occult blood in the feces:
   - a. Definition of occult blood.
   - b. Conditions where occult blood may be present.
   - c. Principles of tests to detect occult blood:
     - (1) Tests: gum guaiac, benzidine, and orthotoluidine.
     - (2) The tests employ oxidizing reactions occurring with the ion in the hemoglobin molecule.
     - (3) Steps in the oxidizing reaction:
   - d. Sensitivity and reliability of the various procedures.
   - e. Collection of the stool specimens.
   - f. Color of the stool specimens:
     - (1) Influence of various foods and medications on the color of stools.

Milk diet.
Meat protein.
Spinach.
Carrots, beets.
Chocolate and cocoa.

High fat diet.
Blood.
Calomel.
Bismuth, iron charcoal.
Barium.

     - (2) Importance of a meat free diet.
   - g. Increase and decrease in amount of stool.
   - h. Properties of stool specimens:
     - (1) Shape and consistency.
     - (2) Gas formation.
     - (3) Mucous or pus.
   - i. "Hematest" (commercial tablet—Ames Company).

Introduction to Laboratory Method

1. Principles of laboratory tests:
   - a. Benzidine.
   - b. Gum guaiac.
   - c. "Hematest" (commercial tablet—Ames Company).
2. Equipment:
   - a. Filter paper tests (benzidine and gum guaiac):
     - (1) Filter paper.
     - (2) Tongue depressor sticks and applicator sticks.
   - b. Test tube methods (benzidine and gum guaiac):
     - (1) Tongue depressor sticks.

- (2) Evaporating dishes.
- (3) 10 ml. graduated cylinders.

c. "Hematest": filter paper.

3. Reagents:
   - a. Filter paper tests (benzidine and gum guaiac):
     - (1) 3 percent hydrogen peroxide.
     - (2) Glacial acetic acid.
     - (3) Benzidine dihydrochloride (powdered).
     - (4) Benzidine solution—unstable and made just before use.
     - (5) 2 percent alcoholic solution of gum guaiac.
     - (6) Working gum guaiac solution—unstable and made just before use.
   - b. Test tube methods (benzidine and gum guaiac): same as for filter paper tests, plus ether.
   - c. "Hematest": commercial "Hematest" tablet.
4. Specimens used—stool specimens obtained from patients.
5. Methods:
   - a. Benzidine—filter paper method:
     - (1) Small amount of feces is smeared on filter paper.
     - (2) Add small amount of freshly prepared benzidine solution to fecal smear on filter paper.
     - (3) Results:
       - (a) Negative—no color.
       - (b) Trace—faint blue forming slowly.
       - (c) Positive—blue appearing almost immediately, never intense.
       - (d) 4 + —immediate intense color.
   - b. Gum guaiac—filter paper method:
     - (1) Small amount of feces is smeared on filter paper with stick.
     - (2) Add 2 drops of glacial acetic acid to feces on filter paper.
     - (3) Add 4 drops of freshly prepared working gum guaiac solution.
     - (4) Results are graded the same as for benzidine.
   - c. "Hematest":
     - (1) Hands, dropper, and working area must be clean and free from traces of blood.
     - (2) Smear a solid specimen of feces on filter paper.
     - (3) Place "Hematest" tablet in center of specimen.
     - (4) Place one drop of water on the tablet, wait 5–10 seconds, and flow a second drop on the tablet so it runs down the sides onto the filter paper.
     - (5) A positive reaction is read on the filter paper within two minutes.
     - (6) Check all positive "Hematest" specimens by running a guaiac and/or benzidine test.
     - (7) Results:
       - (a) Negative—no color appearing on filter paper around the tablet.
       - (b) Positive—area on the filter paper around the tablet turns blue:
         - ● The amount of blood is proportional to the time of appearance and to the intensity of color.
         - ● The color on the tablet is of no significance.
   - d. Benzidine and gum guaiac—test tube method:
     - (1) Soften 1–2 grams of feces in small evaporating dish.
     - (2) Add 8–10 ml. of ether and mix thoroughly.
     - (3) Discard the ether layer.
     - (4) Add ⅓ volume of glacial acetic acid to the remainder of the feces and mix well.
     - (5) Extract the fecal-acetic acid solution with 10 ml. of ether.
     - (6) Into a test tube, mix 1 volume acetic acid-ether-fecal mixture—1 volume benzidine solution—or 1 volume working gum guaiac solution.
     - (7) Results are graded in the same manner as for the filter paper methods.
6. Control:
   - a. Test the reagents by running controls.
   - b. Negative control—filter paper plus reagents.
   - c. Positive control—blood on filter paper plus reagents.

Laboratory Exercise

1. Review and demonstrate filter paper and test tube tests for benzidine gum guaiac, and use of "Hematest" tablets.

2. Student exercises:
   a. On the four unknown stool specimens, do:
      (1) Filter paper tests—benzidine and gum guaiac.
      (2) Test tube tests—benzidine and gum guaiac.
      (3) "Hematest" tablet test.
   b. Prepare fresh working solutions for the tests.
   c. Record your results on Occult Blood—Student Work Sheet.
   d. Include negative and positive controls with your specimens.

Study Questions

1. What is the purpose of doing an occult blood test on a stool specimen?
2. Why is the guaiac test usually used instead of the benzidine test?
3. What is the purpose of running negative and positive controls with each batch of occult blood determinations?
4. The recognition of occult blood in the feces is very important in the diagnosis in what two diseases?
5. In the test tube methods for occult blood, what is the purpose of extracting the feces with ether?
6. What is the function of the glacial acetic acid in the second ether extractions?
7. Which of the following results should be rechecked before giving results to the doctor? Why?

Specimen	Benzidine	Guaiac	Results OK or to be rechecked	Why?
1	4+	neg.		
2	4+	4+		
3	neg.	4+		
4	neg.	trace		
5	trace	trace		

ROUTINE URINALYSIS—STUDENT WORK SHEET

[Sample Student Work Sheets]

Specimen # ______________					
Physical properties	Color				
	Transparency				
Specific Gravity	Reading				
	Urinometer correction				
	Corrected specific grav.				
pH (pH Universal paper)					
Urine sugars (Benedicts qual. test)					
Acetone (Rothera's test)					
Diacetic acid (Gerhardt's test)					
Urine proteins (salt-acetic acid test)					
Urine microscopics:					
Red blood cells (hpf)					
White blood cells (hpf)					
Casts:	Grade (lpf)				
	Identify (hpf)				
Normal crystals (lpf)					
Abnormal crystals (hpf)					
Miscellaneous structures					
Bilirubin (Harrison's test)					
Urobilinogen (Ehrlich's test)					

Student's Name ....

Date ....

GASTRIC ANALYSIS—STUDENT WORK SHEET

Time Amount	Free HCL		Total acid		Boas test	Lactic test
	ml. of 0.1 N NaOH	Degrees of acidity	ml. of 0.1 N NaOH	Degrees of acidity		
Fasting						
20 minute						
40 minute						
60 minute						

OCCULT BLOOD—STUDENT WORK SHEET

Specimen #	Color of specimen	Benzidine		Gum Guaiac		"Hematest"
		Filter paper	Test tube	Filter	Test tube	
Negative control (filter paper + reagents).						
Positive control (blood + filter paper + reag.).						

Student's name: ....

Date: ....

URINE MICROSCOPICS—STUDENT WORK SHEET

Specimen #				
Red blood cells / hpf. Grade 0, occ, 1, 2, 3, 4.				
White blood cells / hpf. Grade 0, occ, 1, 2, 3, 4.				
Casts — Grade / lpf 0, occ, 1, 2, 3, 4.				
Casts — Identify (hpf).				
Normal crystals / lpf. Grade: O, occ, 1, 2, 3, 4. List the kinds seen.				
Miscellaneous.				

Student's name: ....

Date: ....

SULKOWITCH TEST—STUDENT WORK SHEET

Date	Spec. #	Unadjusted urine pH	Adjusted urine pH	Blank (if needed)	Result

PHENOSULPHONPHTHALEIN EXCRETION TEST—STUDENT WORK SHEET (PSP)

Date	Spec. #	Minutes	Urine volume (ml.)	Dilution used	Hellige color		B. & L. color	
					Rdg.	Per cent PSP dye	Per cent PSP dye excreted	Reading

Unit IX Basal Metabolism—Electrocardiography

Suggested Time: 2 weeks; 80 hours.

Unit content

A. Introduction to Basal Metabolic Rates.
B. Calculation of Basal Metabolic Rate Tests.
C. Introduction to Electrocardiography.
D. Performance of EKG Test.
E. Mounting Techniques and Isolation Techniques.

Bibliography

American Heart Association, *Recommendations for Human Blood Pressure*, The Association, New York, N.Y.

Anthony, Catherine P., *Textbook of Anatomy and Physiology*, 5th ed., St. Louis, Mo., C. V. Mosby Co., 1959.

Burch, George E. and Winsor, Travis, *A Primer of Electrocardiography*, 4th ed., Philadelphia, Lea & Febiger, 1960.

EKG Techniques in Electrocardiography Tests, Medical Laboratory Assistant Course, University of Minnesota, Minneapolis, Minn.

Exercise Manual, *Medical Laboratory Assistant Course*, University of Minnesota, Minneapolis, Minn.

Manuals accompanying EKG instruments (i.e. Sanborn, Cambridge, etc.) with instructions for operation and maintenance of machines.

Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. I & II, Baltimore, Md., Williams and Wilkins Co., 1956.

Roe, Joseph H., *Principles of Chemistry*, 9th ed., St. Louis, Mo., C. V. Mosby Co., 1963, Chapter 32.

Techniques in Basal Metabolic Tests, Medical Laboratory Assistant Course, University of Minnesota, Minneapolis, Minn.

Watson, C. J., *Outlines of Internal Medicine*, Part II, 9th ed., Dubuque, Iowa, Wm. C. Brown Co., 1958.

Unit outline

A. Introduction to Basal Metabolic Rates:
  1. Definition of basal metabolic rate.
  2. Factors influencing the BMR:
    a. Age.
    b. Sex.
    c. Height and weight (using the DuBois Surface Area Formula).
    d. Temperature.
    e. Post-absorptive state:
      (1) Definition.
      (2) Respiratory quotient.
    f. Physical repose.
    g. Mental repose.
    h. Medication.
  3. Methods for measuring basal metabolism:
    a. Direct method (colorimeter).
    b. Indirect method (Tissot apparatus).

- c. Modified indirect methods:
  - (1) Benedict-Roth machine.
  - (2) Sanborn Waterless machine.
  - (3) McKesson Metabolator.
- 4. Description of BMR machine:
  - a. Parts of BMR machine.
  - b. Uses for each part.
  - c. Advantages and disadvantages of the BMR machine.
  - d. Precautions in using BMR machine.
- 5. Preparation of the patient:
  - a. Patient date (name, address, age, previous BMR's, post-absorptive state, and physical repose).
  - b. Height and weight of patient.
  - c. Blood pressure.
  - d. Mental repose.
  - e. Temperature and pulse:
    - (1) Procedure for taking temperature and pulse.
    - (2) Temperature correction factor for calculation of accurate BMR.
- 6. Procedure for performance of BMR test:
  - a. Barometric pressure (using barometer).
  - b. Setting up the BMR machine.
  - c. Testing BMR machine for accuracy.
  - d. Connecting the patient to the BMR machine:
    - (1) Explanation and instructions to the patient.
    - (2) Precautions and things to check.
- 7. Care of the BMR machines (after each test, daily, weekly).
- 8. Diseases affecting the BMR:
  - a. Diseases characterized by a high metabolic rate.
  - b. Diseases characterized by a low metabolic rate.

Introduction to Laboratory Method

1. Principle of procedures for taking blood pressures, pulse rates, temperatures, height, weight, barometric pressure, and for use of BMR machine.
2. Equipment and supplies:
   - a. Blood pressure—sphygmomanometer and stethoscope.
   - b. Pulse rate—watch with a second hand.
   - c. Temperature thermometers, tissue wipes, soapy water, and 1:1000 dilution of zephirin chloride (or alcohol).
   - d. Height and weight—scale measuring in kilograms and centimeters for height and weight.
   - e. Barometric pressure—barometer.
   - f. BMR machine.
   - g. Sterilized mouthpieces and noseclips.
   - h. Pencils and rulers.
3. Method:
   - a. Preparation of patient (personal data, height, weight, post-absorptive state, physical repose, mental repose, temperature).
   - b. Setting up the BMR machine and testing for accuracy.
   - c. Connecting the patient to the BMR machine.
   - d. Actual performance of running the BMR test.
   - e. Care of the BMR machine after performance of the test.

Demonstration and Laboratory Exercises

1. Observation of the BMR machine:
   - a. Demonstrate the various parts of the BMR machine and explain the uses of each part.
   - b. Student exercise—Draw a simple diagram of the BMR machine and label all parts.
2. Preparation of patient:
   - a. Demonstrations:
     - (1) Use of the barometer for taking barometric pressure.
     - (2) Use of sphygmomanometer and stethoscope for blood pressures.
     - (3) Correct use and care of thermometers.
     - (4) Taking pulse rate with watch with second hand.
   - b. Student exercises:
     - (1) Take barometric pressure for the day.
     - (2) Work in partners and do the following on each other:
       (a) temperature (b) blood pressure
       (c) pulse rate.

Study Questions

1. What is meant by basal metabolic rate?
2. What is the usage of the following parts

of the BMR machine? (List 6–10 parts found on your own particular BMR machine.)

3. Define: blood pressure, body temperature, pulse rate, systolic pressure, diastolic pressure, respirations.
4. What is the percent error in BMR due to rise in temperature?
5. What are some defects in the instruments used for taking blood pressures which would result in an inaccurate blood pressure?
6. What are the normal values for the following:
   a. Temperature, pulse rate, blood pressure.
   b. BMR.

B. Calculation of Basal Metabolic Rate Tests:

1. Formulas for calculating BMR:
   a. Gas factor.
   b. Respiratory quotient.
   c. Dubois Surface Area Formula.
2. Calculations:
   a. Using the liters of oxygen the patient used in a specific time interval as obtained by running the test and converting to cal./sq. m./hr.
   b. Comparison of calories/sq. meter/hour with the average normal for age, sex, and living environment giving the answer in terms of percent.
   c. Correction for patient's temperature if necessary.
3. Determination of a satisfactory BMR:
   a. Evaluation of patient data (temperature, physical and mental repose, post-absorptive state).
   b. Evaluation of BMR test with duplicate runs.
4. Cutting and mounting of BMR tracing with proper recording of data.
5. Review of BMR terminology:
   a. Basal metabolic rate.
   b. Blood pressure (systolic and diastolic).
   c. Barometric pressure.
   d. Vital capacities.
   e. Tidal air.
   f. Minute volume.

Demonstration and Laboratory Exercises

1. Interview and preparation of the patient:
   a. Demonstrate proper technique for interviewing and obtaining personal data from the patient (name, age, post-absorptive state, mental and physical repose, temperature, blood pressure, pulse rate, height, weight) and obtaining barometric pressure.
   b. Student exercises:
      (1) Work as partners and do the following on each other:
         (a) personal data (b) temperature
         (c) blood pressure (d) pulse rate.
      (2) Take blood pressure of two other people.
      (3) Obtain barometric pressure from barometer.
2. Performance of BMR test—(students work as partners):
   a. Set up the BMR machine, check for accuracy, connect patient to machine and run a timed BMR test.
   b. Save recording and tape on Student Work Sheet (for calculation at a later time).
   c. Record all data on Student Work Sheet.
3. Calculation of BMR test:
   a. Review of the calculations for the BMR test.
   b. Student exercise:
      (1) Using the data from Exercise 2, calculate the BMR of your patient.
      (2) Record all data on Student Work Sheet.
      (3) Also calculate the tidal air and minute volume for the exercise.
4. Calculation exercises. Complete calculations of assumed patients with given date (age, height, weight, temperature, barometric pressure, spirometer temperature, and liters of oxygen/-timed interval), record on Student Work Sheets.

Study Questions

1. During the actual running of the BMR test, what are the 3 factors noted?
2. Briefly describe the following diseases, giving their clinical findings, symptoms, and manifestations:
   a. myxedema.

    b. hyperthyroidism.

3. Name 3 diseases in which the BMR may be elevated.
4. Name 3 diseases in which the BMR may be low.
5. List 8 factors which would affect the BMR.
6. What kind of a result (high or low) would you expect in the BMR if there was a leak in the machine?
7. What is the criterion for drawing the oxygen slope line?
8. As a laboratory assistant, what is your prime duty toward the patient in running a BMR test? (It is also an important factor influencing the BMR.)
9. Define:
    - a. exophthalmus.
    - b. post-absorptive state.
    - c. respiratory quotient.
    - d. physical repose.
    - e. mental repose.
    - f. tidal air.
    - g. minute volume.
    - h. barometric pressure.
    - i. vital capacity.
10. If your patient had a temperature of 99.9°F., calculate the change in the BMR.

Student's name ....

STUDENT WORK SHEET FOR BMR EXERCISES

Patient's name: .... **Date:** .... **Time:** ....

Address: .... **Previous tests:** .... **Where:** ....

8–10 hr. rest: .... **No food or drink since 7 p.m. evening before** ....

Age: .... **Sex:** .... **Height:** .... **Weight:** ....

Blood pressure: .... **Barometric pressure:** ....

Temperature: .... **Pulse rate:** ....

	Pulse	Respirations	Spirometer temp.	Liters 0_2 consumed time
Test # 1				
Test # 2				

Calculations	Test # 1	Test # 2
Surface area (sp. m.)		
Liters of 0_2 / hour		
Cal./sq. m./hr		
Normal value (cal/sq.m./hr.)		
% BMR (minus or plus)		
Temperature correction		
% BMR (corrected for temp)		
Tidal air		
Minute volume		
Results of test (repeat, satisfactory, unsatisfactory)		

—Cut and tape BMR test in this space—

Student name: ....

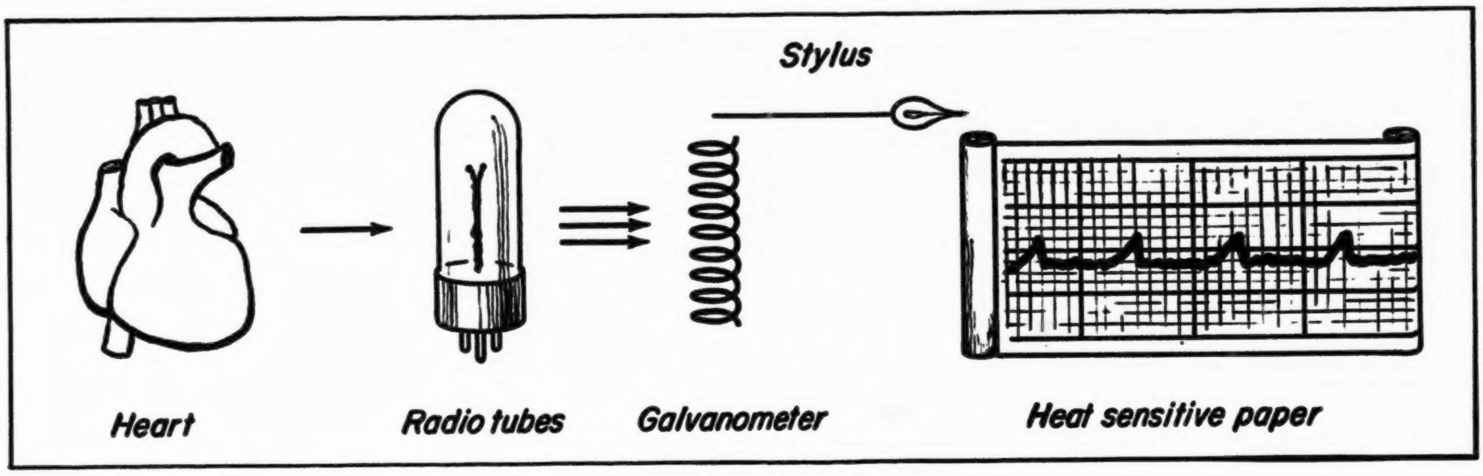
BMR CALCULATIONS—STUDENT WORK SHEET

	Patient # 1	Patient # 2	Patient # 3	Patient # 4	Patient # 5	Patient # 6	Patient # 7	Patient # 8
Name	Mrs. Brown	Jane Smith	Mr. Large	Miss Small	Mr. Grey	Bob Jones	Mary Doe	Miss Roe
Age	32 years	5 years	42 years	59 years	61 yrs.	12 years	18 years	23 years
Weight	69 kg.	26 kg.	97 kg.	44.3 kg.	63.1 kg.	40 kg.	55.4 kg.	58 kg.
Height	159 cm.	100 cm.	182 cm.	162.5 cm.	147 cm.	142 cm.	164 cm.	168 cm.
Temperature	98.8°F.	99.6°F.	98°F.	98.6° F.	100°F.	98°F.	98.6°F.	98.8°F.
Barometric Pressure	751 mmHg	750 mmHg	745 mmHg	745 mmHg	740 mmHg	749 mmHg	750 mmHg	755 mmHg
Spirometric Temperature	22°C	23°C.	21°C.	21.5°C.	23°C.	21°C.	20°C.	22°C.
Liters O_2/6 minutes	1.12	0.65	2.20	1.50	1.33	1.20	1.30	1.41
Surface Area (sq.m.)								
Liters O_2/hour								
Cal./sq.m./hour								
Cal./hour patient used								
Normal Valve (cal/sq.m./hr)								
% BMR (minus or plus)								
Temperature Correction								
% BMR (corrected for temp.)								

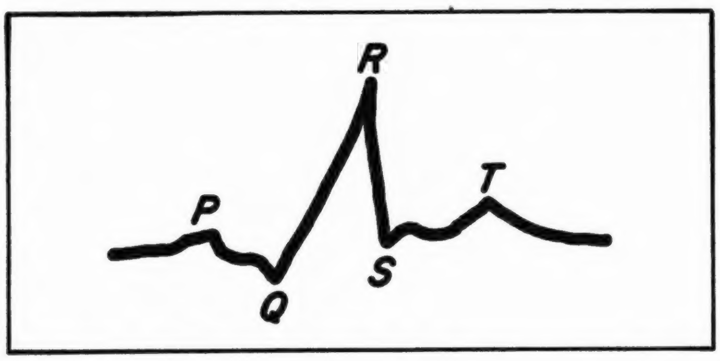
C. Introduction to Electrocardiography:

1. Definition of electrocardiography, electrocardiogram, electrocardiograph.
2. General principle of the EKG machine (Cambridge, Sanborn, etc.):
   - a. Parts.
   - b. Usage of the different parts.
   - c. Diagram of the mechanism of the machine.

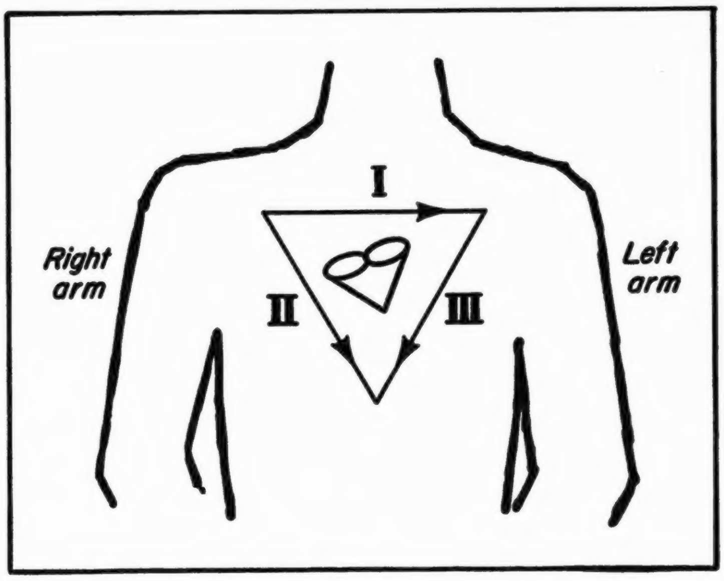
Example:



3. Heart:
   - a. Definition.
   - b. Diagram of chambers of heart and main vessels.
   - c. Functions of each chamber of the heart (RA, RV, LA, LV).
   - d. Diagram of circulation of blood.
   - e. Contraction of the chambers of the heart.
   - f. Explanation of cardiac waves:
     - (1) "P" wave.
     - (2) "QRS" complex.
     - (3) "T" wave.



   - g. Interpretation of an electrocardiogram:
     - (1) Normal electrocardiogram pattern.
     - (2) Abnormalities in the electrocardiogram pattern.
4. Leads (limb and chest):
   - a. Limb leads:
     - (1) Bipolar limb leads (I, II, and III).



Lead I —right arm to left leg.
Lead II —right arm to left leg.
Lead III—left arm to left leg.
Right leg acts as a ground.

     - (2) Unipolar limb leads or augmented vector (AV) leads.
       - (a) Lead AVR—right arm potential.
       - (b) Lead AVL—left arm potential.
       - (c) Lead AVF—left leg potential.
   - b. Chest leads (V):
     - (1) Adult (show diagram):
       - (a) Lead V_1—fourth intercostal space at *right* border of sternum.

      (b) Lead V_2—fourth intercostal space at *left* sternal border.
      (c) Lead V_3—midway between V_1 and V_2.
      (d) Lead V_4—at outer border of apex beat area or in midclavicular line at the fifth intercostal space.
      (e) Lead V_5—same level as V_4, in anterior axillary line.
      (f) Lead V_6—same level as V_4 and V_5 in mid-axillary line.
    (2) Variation in children.
    (3) Lead marking system (code).

D. Performance of EKG Test:

1. Standardization of EKG machine:
   a. Time standardization (horizontal tracing).
   b. Voltage standardization (vertical tracing).
   c. Voltage standardization at 0.5 centimeters.
2. Preparation of patient:
   a. Explanation of EKG test to patient.
   b. EKG electrodes:
    (1) Usage and purpose of electrodes.
    (2) Application of electrodes to patient:
      (a) Limb electrodes.
      (b) Chest electrodes.
      (c) Precautions to take in applying electrodes.
   c. Attaching the EKG patient cable connections.
3. Operation of EKG machine (see manual accompanying the machine):
   a. Connecting EKG machine.
   b. Proper grounding.
   c. Parts and usage of machine.
   d. Standardization of machine.
   e. Taking the limb leads (1, 2, 3, AVR, AVL, and AVF).
   f. Taking the chest leads (V_1, V_2, V_3, V_4, V_5, and V_6).
   g. Completing the EKG test.
4. Care of the EKG machines:
   a. Care after each EKG test.
   b. Daily EKG duties.
5. Difficulties and remedies in taking EKG:
   a. Somatic tremor.
   b. Alternating current (AC) interference.
   c. Wandering baseline.
   d. Jittery baseline.
   e. Poorly defined baseline.
6. Variations in technique for taking EKG:
   a. Infants.
   b. Infectious diseases.
   c. Master's "2 step" test (exercise EKG).

Introduction to Laboratory Method

1. Review of parts and usage of the EKG machine.
2. Equipment and supplies:
   a. EKG machines.
   b. EKG electrodes and patient cable cord.
   c. EKG electrode jelly.

Laboratory Exercises

1. Operation of the EKG machine (standardization and limb leads):
   a. Demonstrations:
    (1) Demonstrate the various parts of the EKG machine and explain the uses of each part.
    (2) Demonstrate proper application of limb lead electrodes to patient.
   b. Student exercise—Work in partners and do the following:
    (1) Standardize the EKG machine.
    (2) Do the six limb leads on partner and save tracing. (Tracings are mounted at a later laboratory period.)
2. Performance of EKG Test:
   a. Chest leads—Demonstrate the positions and placement of electrodes on chest of patient.
   b. Students—Work in partners and do the following:
    (1) Standardize the EKG machine.
    (2) Do the six limb leads and the six chest leads on partner and save tracing to be mounted at a later laboratory period.
   c. Standardization at 0.5 cm.
    (1) Demonstrate technique of standardization at 0.5 centimeter.
    (2) Student exercise—Work in partners and do the following:
      (a) Standardize the EKG machine in the usual manner.
      (b) Do the six limb leads and the six chest leads on partner.

(c) Assume that lead V_3 has to be cut down to a standardization of 0.5 centimeter because its peaks go beyond the edge of the ruled area. Cut down lead V_3 according to directions and return lead V_4 to full standardization.

(d) Save tracing to be mounted at a later laboratory period.

Study Questions

1. Define the following:
   a. electrocardiograph.
   b. electrocardiogram.
2. Draw a diagram of the heart and show how the blood circulates through the heart.
3. Name the 4 chambers of the heart and the functions of each chamber.
4. Trace the route of the heart voltages, from the patient to the recording tape.
5. What is the difference between unipolar and bipolar leads?
6. To what phase of cardiac circulation does each wave of the electrocardiogram correspond?
7. What particular potential does each of the limb leads represent?
8. What is the purpose of grounding the machine?
9. Why is every tracing standardized?
10. What facts must be recorded on every electrocardiogram?
11. How fast does the paper in an electrocardiograph machine move?
12. a. How do you regulate the width of the baseline of the electrocardiogram?
    b. How wide should the baseline be?
13. a. When would you find the need to standardize at 0.5 centimeter?
    b. Briefly describe the technique you would use to standardize at 0.5 cm.

E. Mounting Techniques and Isolation Techniques.

1. Mounting techniques.
   a. Technique involved in mounting EKG tracings.
   b. Proper labeling and identification of EKG tracings.
2. Isolation techniques.
   a. Precautions involved in doing EKG on patients in isolation.
   b. Technique involved in doing EKG in isolation.
      (1) Personal technique to avoid contamination.
      (2) Technique for operation of EKG machine.
      (3) Special equipment needed.
   c. Clean-up of operator and EKG machine after doing EKG in isolation.

Laboratory Exercises

1. Work in partners and do the following:
   a. Do an EKG as would be done normally on a patient.
   b. Cut and mount tracing on the EKG student work sheets.
   c. Be sure that tracings are correctly labeled.
   d. Hand in all EKG mountings.
2. Change the chart paper in EKG machine.
3. Change the dessicant in EKG machine.
4. Have an instructor check EKG machine.
5. Students should be graded on operation and knowledge of the machine.

Study Questions

1. What is the universal standard for voltage standardization?
2. What would you do if a patient had one leg amputated?
3. Draw an example of the following:
   a. Somatic tremor.
   b. "AC" interference.
   c. Wandering baseline.
4. What does the electrode jelly consist of? What is the purpose of each ingredient?
5. What is the location of:
   a. Lead V_1
   b. Lead V_4
   c. Lead V_6
6. How would you remedy the following:
   a. "AC" interference.
   b. Wandering baseline.
   c. Somatic tremor.
   d. Poorly defined baseline.
   e. Jittery baseline.
7. What is Lead V_4R? When is it taken? What does it detect?

8. What is the purpose of the Master's test?
   b. Why must a physician be present during the Master's test?

9. Briefly describe the isolation technique.

10. Briefly describe the technique you would use in doing an EKG on infants.

EKG STUDENT WORK SHEET

Patient: ....

Date: ....

Time: ....

Properly labeled standardization

Lead I   Lead II   Lead III   Lead AVR

Lead AVL   Lead AVF   Lead V_1 Lead V_2

Lead V_3 Lead V_4 Lead V_5 Lead V_6

Student name: ....

APPENDIXES

APPENDIX A

Requirements of the Board of Certified Laboratory Assistants of the American Society of Clinical Pathologists and the American Society of Medical Technologists

(Extracted from Guidebook and Essentials for an Approved School of Laboratory Assistants, May 1964)*

The program for training of laboratory assistants is not to be construed as being a program to train replacements or substitutes for medical technologists, nor is it intended at any time to duplicate the training program for medical technologists. The sponsoring organizations recognize the registered ASCP medical technologist as the primary and pivotal laboratory worker through whom the laboratory director sets standards for quality performance.

The scope of the training program in schools approved for training laboratory assistants must be adjusted to the limits imposed by the required academic background of the trainee. Laboratory assistants trained in these schools are to work at all times under the supervision of qualified registered ASCP medical technologists and pathologists or other qualified physicians and never as independent practitioners.

The CLA Board has established the following standards for this training:

A. Administration:

1. Acceptable schools for training certified laboratory assistants may be conducted by approved medical schools, hospitals, or other acceptable laboratories, suitably organized in accordance with present educational standards. Acceptable schools must be separate from A.M.A.-Approved Schools of Medical Technology, and although both types of schools may be operated in selected large institutions, there must be complete physical separation of instruction of the two types of students.

2. All training shall be under competent medical control.

3. Resources for continued operation should be insured through regular budgets, gifts, or endowment, but not entirely through student fees.

B. Organization:

1. Adequate space, light and modern equipment should be provided in the laboratory. A library containing up-to-date references, texts and scientific periodicals pertaining to clinical laboratory work should be maintained or be readily accessible.

2. Satisfactory record systems should be provided for all work carried on in the laboratory, with monthly and annual compilations.

3. Transcripts of high school credits and other credentials must be available. An acceptable school keeps records of each student's attendance and grades, and it is also recommended that the type of tests performed be recorded. The school should have a synopsis of the complete curriculum on file, including the rotation of assignments, outlining of instruction supplied by the laboratory, and a list of the prepared specimens used to augment the student's experience.

4. At least two students should be enrolled in each class. Classes should not be started more frequently than semi-annually except under especially approved circumstances.

C. Faculty:

1. The school should have a competent teaching staff. The director must be a graduate in medicine who is certified by the American Board of Pathology or who has had training and experience in clinical pathology acceptable to the CLA Board. He shall take part in and be responsible for the actual conduct of the training course. He shall be in daily attendance for sufficient time to supervise properly the laboratory work and teaching.

2. In laboratory practice, enrollment may not exceed two students to each member of the teaching staff. The staff must include not less than one instructor whose duties include supervising and teaching program and who possesses a bachelor's degree, is a registered medical technologist (ASCP) and who has had three years of experience or its equivalent. A technologist instructor should be a registered medi-

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* Further information is available from the Secretary, Board of Certified Laboratory Assistants, 9500 South California Avenue, Evergreen Park, Ill. 60642.

cal technologist (ASCP) or equivalently qualified, and preferably have had at least one year of experience.

D. Prerequisites for admission:

1. Primary educational requirement for admission is graduation from a high school accredited by the appropriate regional accrediting agency. It is recommended that applicants have demonstrated ability and interest in science and mathematics.

2. Certification of equivalent training may, at the discretion of the Board, be accepted in lieu of this requirement.

E. Curriculum:

1. The course of training must be at least 12 months in duration and should be uninterrupted. A student shall be required to complete 40–44 hours of laboratory training per week.

2. A minimum of 100 didactic instruction hours is required either in the form of tutorial type organized instruction if class enrollment is less than five students, or as formal lectures if enrollment is five or more. Stress is to be placed on technical performance and the sources and detection of errors rather than pure theory.

3. Ten (10) weeks of training must be devoted to "Orientation to the Clinical Laboratory" and include basic orientation in: professional adjustments, relations and organizations; medical ethics and conduct; medical terminology; laboratory records; basic anatomy and physiology; handling, identifying, and care of laboratory equipment; aseptic technique and methods of sterilization; basic laboratory mathematics; preparation of basic laboratory solutions and media; basic elements of quality control; handling of histologic and cytologic specimens; instruction in blood collecting techniques; introduction to basic hematology, serology, blood banking, urinalysis, basal metabolism, and electrocardiography.

4. The sequence of instruction during the remainder of the course may be at the discretion of the director. It shall include basic technical instruction in bacteriology, serology and parasitology—8 weeks; hematology—10 weeks; clinical chemistry—10 weeks; blood banking—6 weeks; routine analysis—6 weeks; and BMR-EKG—2 weeks.

5. The instruction should follow a planned outline and include text assignments, lectures, discussions, demonstrations, supervised practice, practical examinations, and quizzes, both oral and written. Deviations from the plan may be authorized by the Board.

F. Clinical material:

1. Each student should receive practice training, adequate in kind and amount, under competent supervision. Participating hospitals and laboratories should be accredited or be otherwise acceptable to the Council on Medical Education of the American Medical Association and/or the Board of Certified Laboratory Assistants.

2. Approved schools should have available laboratory material equivalent to that provided by a hospital of 100 beds and 3,000 yearly admissions and a minimum of 50,000 tests a year with a distribution of clinical material sufficient to provide adequate technical training in the various laboratory divisions.

G. Ethics:

1. Excessive fees and commercial advertising should be considered unethical.

2. Approved schools shall not be operated for profit and student fees shall not exceed the cost of books, supplies, a nominal allowance for breakage, or when necessary, other operational costs.

3. Schools must clearly indicate type and level of training.

4. Schools conducted primarily for the purpose of substituting students for paid medical technologists will not be considered for approval:

a. Student should not assume the responsibility or take the place of a qualified medical technologist or certified laboratory assistant.

b. The provision of room, board and maintenance, or a minimal monetary allowance in lieu thereof, are acceptable. Monetary stipends in excess of this allowance are discouraged.

c. The staff of medical technologists and certified laboratory assistants should be adequate to accomplish the work of the department independent of the presence of students.

d. Any indication of exploitation of students will result in withholding or retraction of approval of a school.

5. The certified laboratory assistant shall be required to adhere to the following Code of Ethics:

"Fully cognizant of my responsibilities as a certified laboratory assistant, I affirm my willingness to discharge my duties with accuracy, thoughtfulness, and care.

"Realizing that the knowledge obtained concerning patients in the course of my work must be treated as confidential, I hold inviolate the confidence (trust) placed in me by the patient and by my supervisor and physician.

"Recognizing the limitations of my duties, I shall work at all times under the supervision of a qualified doctor of medicine and/or a medical technologist, who is registered by the American Society of Clinical Pathologists and who abides by the Code of Ethics of that profession.

"The Code of Ethics shall be consistent with the Code of Ethics of the American Medical Association."

H. Health:

1. Applicants shall be required to submit evidence of good health, and a report of a medical examination including a roentgen examination of the chest shall be a part of the student's record.

2. Provision should be made for medical care and hospitalization, when necessary, for a reasonable length of time.

3. Every precaution should be taken to prevent any health hazard in the laboratory. Any injuries on duty should be taken care of at once as an emergency.

I. Admission to the Approved List:

1. At present, application for approval of a school may be sent to Secretary, Board of Certified Laboratory Assistants, 445 North Lake Shore Drive, Chicago, Ill. 60611. Forms will be supplied for this purpose on request and should be completed by the director of the laboratory requesting approval. Inquiries regarding certification of qualified laboratory assistants should be similarly addressed.

2. Schools applying for approval shall be subject to an initial inspection, and approved schools shall be subject to periodic reinspection by teams composed of representatives of the American Society of Clinical Pathologists and the American Society of Medical Technologists. An annual report on a form approved by the Board will be required of all approved schools. Continued approval shall be based on the fulfillment of the standards for the training of Certified Laboratory Assistants.

a. If on inspection an approved school is found to be deficient in its educational program, it shall be so notified and if the deficiency is not corrected within one year, approval shall be withdrawn.

b. If a school has not functioned for two years, it shall be notified that if it is not reactivated within the next year, it shall be dropped from the list of approved schools.

3. Approval of a school may be revoked by the Board for cause at any time.

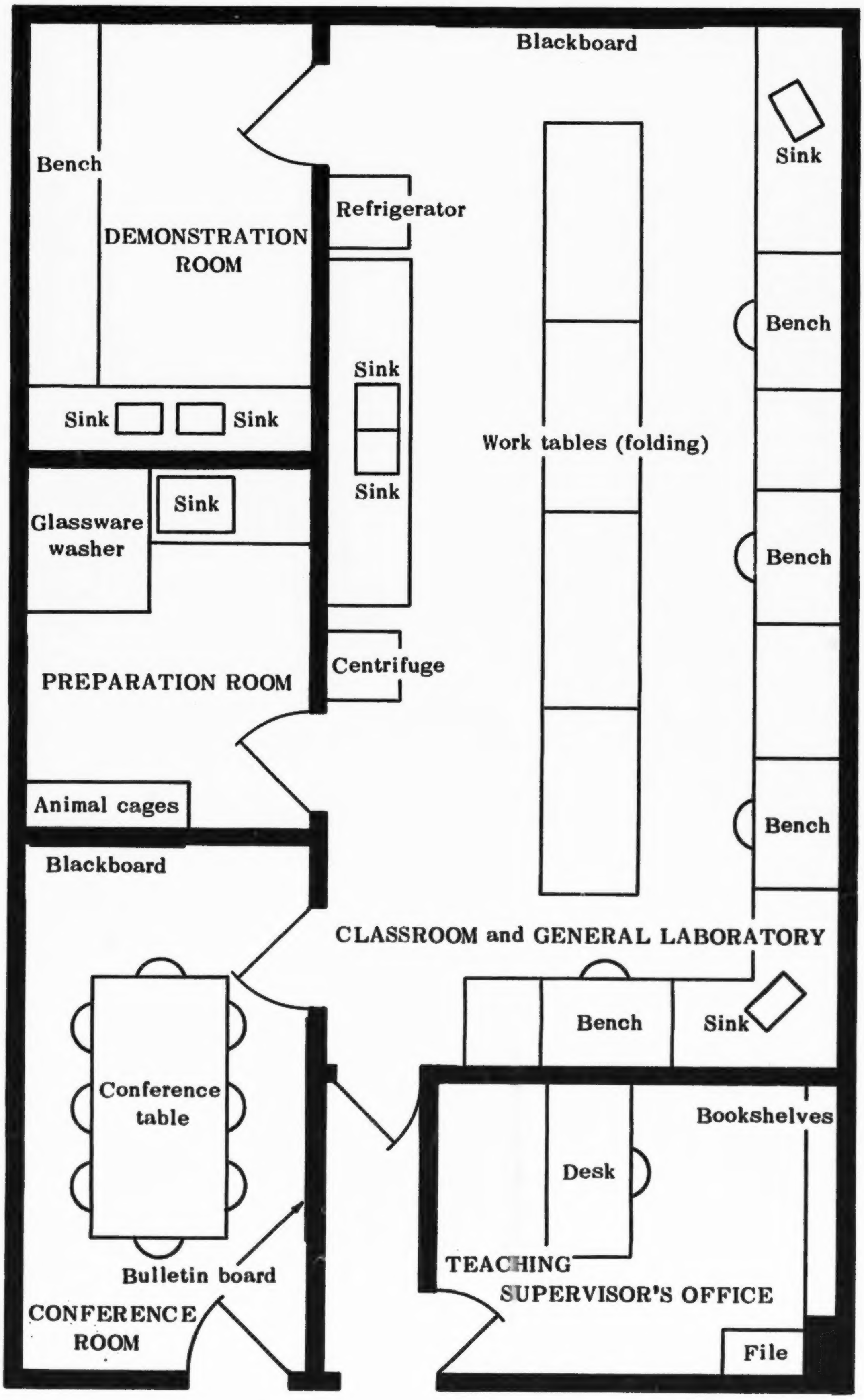
4. Approved schools shall notify the CLA Board whenever a change occurs in the directorship of the school.

a. If there is a change, information on qualifications of the new pathologist-director must be submitted in duplicate to the Board as soon as possible. If the new director's credentials are in order, the school's approval will be continued.

b. If there is an extended interval during which there is no director, the students already enrolled will be permitted to complete the course but no new students may be enrolled until a pathologist-director is appointed and approved.

APPENDIX B

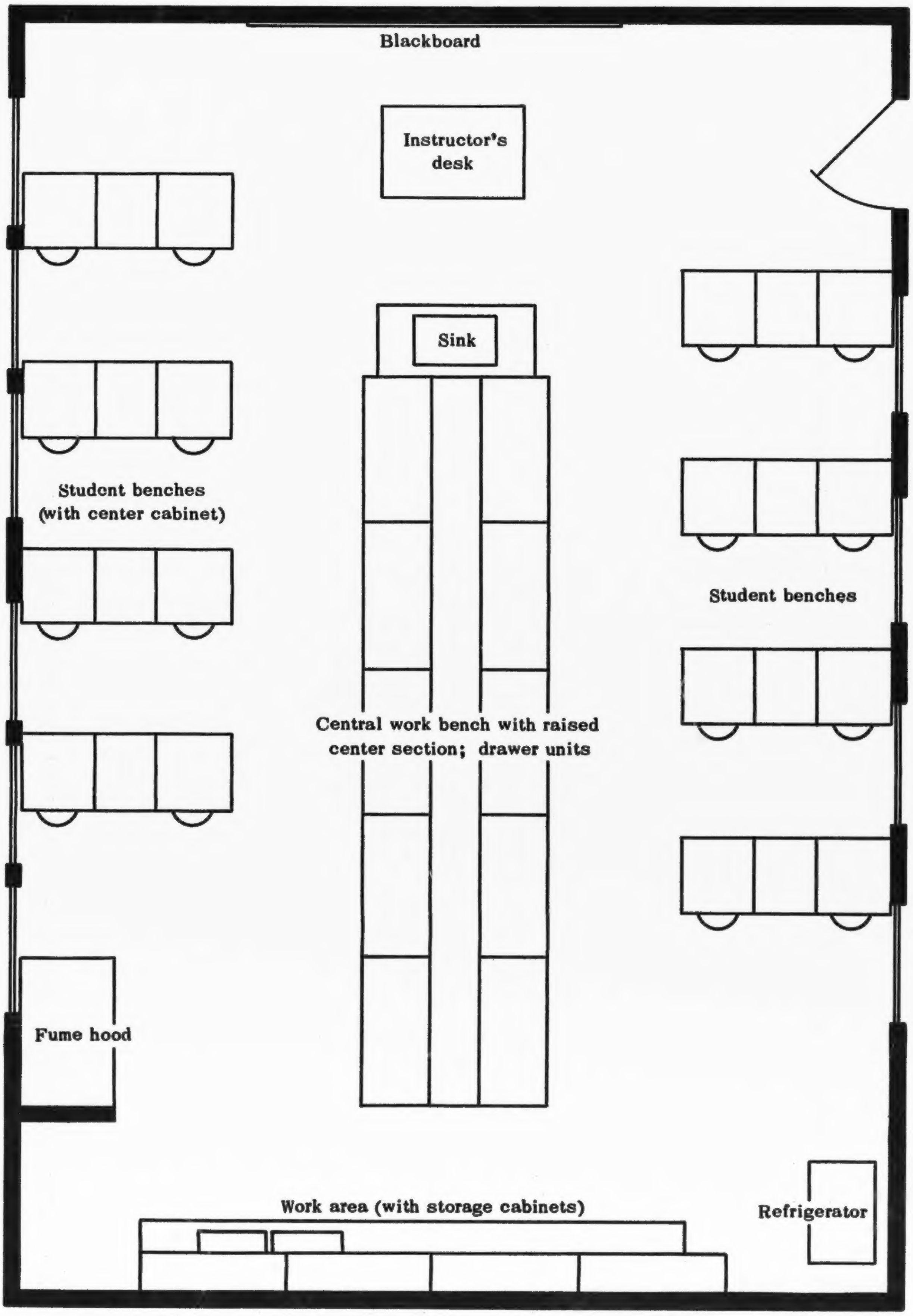
Training Laboratory in Hospital Program



Blackboard
Sink
Bench
Refrigerator
DEMONSTRATION ROOM
Bench
Sink
Sink
Sink
Work tables (folding)
Sink
Sink
Glassware washer
Bench
Centrifuge
PREPARATION ROOM
Animal cages
Bench
Blackboard
CLASSROOM and GENERAL LABORATORY
Bench
Sink
Conference table
Bookshelves
Desk
Bulletin board
TEACHING SUPERVISOR'S OFFICE
CONFERENCE ROOM
File
Corridor
SCALE: 3/16″=1′

APPENDIX C

Training Laboratory in Vocational School


Blackboard
Instructor's desk
Sink
Student benches (with center cabinet)
Student benches
Central work bench with raised center section; drawer units
Fume hood
Work area (with storage cabinets)
Refrigerator

SCALE: 1/4″=1′

APPENDIX D

Equipment for Training Program

A. Suggested Non-Expendable Items of Equipment

Analytical balance.
Artificial arm (for venipuncture).
Autoclave.
Automatic pipette washer.
Balance—triple beam.
Barometer.
BMR machine of standard make and quality.
Bunsen burner—one for every 2 students.
Burette stand, rod and clamp—one for every 2 students.
Centrifuge:
  Floor model—one for every 12–15 students.
  Table model—one for every 6 students.
  Micro-hemotocrit and reader.
Chemistry hood.
Differential counter—one for each student.
Distilling apparatus.
EKG machine on standard make and quality.
Hematology pipette washer—one for every 6 students.
Incubator, standard laboratory model.
Interval timer.
Microscope and lamp—one binocular per student.
NPN heater (6 unit)—one for every 3 students.
Pipette shaker—one per student.
Polyethylene pipette jars.
Racks:
  Filtering rack—one for every 3 students.
  Sedimentation rack (6 place)—one for every 6 students.
  Staining rack—one for every 6 students.
  Test tube rack—two per student.
Refrigerator.
Rotator—for VDRL's—one for 10 students.
Spectrophotometer:—One for every 6 students. One each of the most common types used in the area if possible.
Stop watch—one for each student.
Thermometer—one for every 2 students.
Utility oven.
View box—one for every 2 students.
Water bath—one medium size for every 6 students.

B. Suggested Expendable Equipment Items (average number per student, except as otherwise noted)

Beakers:	
50 ml.	3
100 ml.	2
150 ml.	1
250 ml.	1
400 ml.	1
600 ml.	1
Flasks—Volumetric with stopper:	
50 ml.	3
100 ml.	4
200 ml.	1
500 ml.	1
1000 ml. (per 2 students)	1
Erlenmeyer:	
25 ml.	10
50 ml.	2
125 ml.	3
250 ml. (per 2 students)	1
500 ml. (per 2 students)	1
1000 ml. (per 2 students)	1
Test tubes:	
13 x 100 mm.	12
15 x 125 mm.	18
16 x 150 mm.	6
10 x 75 mm.	24
Centrifuge tubes:	
graduated	6
plain	12
Cuvettes for colorimeter	12
Cylinders, graduated:	
10 ml.	2
25 ml.	2
100 ml.	1
500 ml. (per 2 students)	1
1000 ml. (per 2 students)	1
Sedimentation Rate tubes	2
Curvettes for colorimeter	12

Bottles—Reagent with ground glass stoppers:
  125 ml.
  250 ml.
  500 ml.
  1000 ml.
  brown (500 ml.)
  dropping

(Bottles should be available in sufficient quantities for each student to use in preparation and storage of reagents, stains, solutions, etc. Quantities are not listed but suggested kinds and sizes are listed.)

VDRL Antigen	2
Pipettes—(per 2 students):	
Volumetric:	
1 ml.	18
2 ml.	12
3 ml.	24
4 ml.	10
5 ml.	12
10 ml.	6
Measuring—(Mohr)	
2 ml.	4
1 ml.	6
5 ml.	4
10 ml.	12
Ostwald—1 ml.	12

Blood Counting:	
RBC	20
WBC	20
Hemaglobin	20
Burettes:	
Automatic burette	One per class
Burette with stopcock	One per student
Miscellaneous:	
Foline—Wu Sugar tubes	5
NPN tubes	4
Urinometers with cylinder	2
Esbach Albuminometer (per 2 students)	1
Funnels—65 mm. dia.	10
Counting chambers (with several extra in case of breakage)	1

APPENDIX E

Suggested Supplies

Miscellaneous:
- Filter paper
- Lens paper
- Glass tubing
- Rubber tubing
- Glass slides
- Cover slips
- Glass stirring rods
- Wooden applicator sticks
- Syringes
- Needles
- Tourniquets
- Stop-cock grease
- First Aid kit
- Towels

Hematology:
- Capillary tubes
- Blotting paper
- Blades for finger pricks
- Mouth pieces and tubing

Chemistry:
- Weighing paper
- Forceps
- Tongs
- Spatulas

Urinalysis:
- pH paper
- Urine cartons or bottles

Blood Bank:
- Polyethylene bottles (wash bottles)

Dishwashing:
- Brushes
- Acid cleaning solution
- Detergent
- Rubber gloves
- Wire baskets

Paper Supplies:
- Scratch pads
- Semi-log graph paper
- Report sheets
- Rulers, scissors, stapler, etc.
- China marking pencils
- Glass marking pencils (diamond point)
- Labeling tape
- Mailing cartons from State Health Department Laboratory

List of reagents and chemicals should be prepared from procedures to be used in the teaching program.

APPENDIX F

NAME: **TALLY OF TESTS PERFORMED** YEAR:

T	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	BACTERIOLOGY
																																Acid fast stain
																																Gram stain
																																Sensitivities
																																Ova & Parasites
																																Neutral Fat
																																Occult Blood
																																BLOOD BANK
																																ABO Grouping
																																Rh Factor
																																Donors
																																BMR
																																CHEMISTRY
																																Amylase
																																Bilirubin
																																BSP
																																BUN
																																Ceph floc
																																Creatinine
																																Diagnex
																																Glucose
																																Total Protein
																																Thymol
																																Uric Acid
																																Albumin
																																CSF Protein
																																Gastric Analysis
																																EKG
																																HEMATOLOGY
																																WBC
																																Hematocrit
																																Hemoglobin
																																Differential
																																RBC
																																Bleeding Time
																																Coag. Time, L—W
																																Coag. Time, Cap.
																																Sed Rate
																																Prothrombin Time
																																CSF Cell Count
																																L. E. Prep.
																																SEROLOGY
																																VDRL
																																Heterophile
																																URINALYSIS
																																Routine
																																Bile
																																Fishberg
																																PSP
																																Water Load
																																NOTEBOOK INSPECTED

APPENDIX G

TALLY OF TEST RESULTS

Name of test	Result—Unknown	Standard result	Date

APPENDIX H

WEEKLY REPORT OF LABORATORY PROCEDURES AND ASSIGNMENTS COMPLETED

Student: Number **Name** **Week number:**

Lab assignment

Lecture attendance: Present: **Absent:**

Demonstration attendance: Present: **Absent:**

Practice sessions missed:

Procedures completed in laboratory	Monday		Tuesday		Wednesday		Thursday		Friday		Saturday		Sunday	
	No.	Init.*	No.	Init.	No.	Init.	No.	Init.	No.	Init.	No.	Init.	No.	Init.

Reading assignments completed:

Miscellaneous:

* This form is to be completed by the student, initialled by the responsible instructor, and turned in before starting the next week's classes.	Student. Approved	Complete

APPENDIX I

MONTHLY REPORT OF TRAINING

Name .... Month of .... 19..........
(Last) (First) (Middle initial)

Hours brought forward	Unit No.	Unit	Time																							Total Monthly Hours	Hours to date	Grade
	1	Orientation																										
	2	Bacteriology																										
	3	Serology																										
	4	Parasitology																										
	5	Hematology																										
	6	Clinical chemistry																										
	7	Blood banking																										
	8	Routine analysis																										
	9	BMR—EKG																										

Tardiness .... Attitude .... Adjustment to .... Progress—Quantity of work .... other ....

Absence .... Conduct .... Training situation .... Progress—Quality of work ....

KEY TO RATING

O—Outstanding.

S—Satisfactory.

U—Unsatisfactory. .... Date .... 19..........
Student's signature Instructor's signature

APPENDIX J

STUDENT RECORD

(By Departments)

Student ....

Department .... Dates: From .... To............................

	Very good	Good	Fair	Poor
Intelligence				
Perseverance				
Initiative				
Ability to follow directions				
Organizing ability				
Willingness to accept responsibility				
Judgment				
Accuracy				
Speed				
Spirit of cooperation				
Manner with patients				
Personal appearance				

Test grade	Evaluation grade
A—90–100	Very good—4
B—80–90	Good—3
C—70–80	Fair—2
F—Below 70	Poor—1

Tests and quizzes

Date	Grade

Evaluation grade: ....

Comprehensive: ....

Final grade on course: ....

Remarks and impressions: ....

....

....

....

....

....

Signature of instructor

APPENDIX K

STUDENT PROGRESS REPORT

Instructors please grade as follows:

1. Whenever possible use letters indicating: A—Very Good; B—Good; C—Satisfactory; D—Unsatisfactory or Poor.
2. Where a letter grade seems unsufficient, supplement with remarks.

Name of Student

Instructor

GROUP I. WORK RATING:

1. Accuracy (quality of work)
2. Speed (without sacrifice to accuracy)
3. Dependability
4. Sense of responsibility (toward learning, performing tests assigned, and conduct)
5. Organizing ability
6. Economy in use of materials, time, energy, etc.
7. Technique in general
8. Consistency and uniformity (consider replicates repeated standards and unknowns)
9. Initiative and judgment
10. Attention to details
11. Completes work on time
12. Dexterity (ability to work with hands and to handle equipment)
13. Technical competence (resourceful, orderly, systematic, knows principles underlying technique, etc.)

GROUP II: MISCELLANEOUS

1. Intelligence (uses his head)
2. Industry (makes use of spare time to good advantage)
3. Application to study
4. Background in basic sciences
5. Ability to learn
6. Desire to learn
7. Scientific curiosity (extra reading—interest in new procedures)
8. Willingness to seek advice and help
9. Improvement
10. Interest in the subject (above average, average, or indifferent)
11. Inspiration of confidence (poise, self-control, sincerity, etc.)
12. Mental alertness (accepts results blindly and without question or realizes and corrects errors)

GROUP III: PERSONALITY RATING:

1. Cooperativeness (ability to get along with fellow-workers)
2. Attitude toward fellow-workers
3. Attitude toward staff
4. Attitude toward profession
5. Professional conduct (ethics, mannerisms, etc.)
6. Work habits (neatness, planning ahead, etc.)
7. Personal appearance
8. Willingness to accept suggestions and criticism
9. Punctuality
10. Courteousness
11. Tact
12. Adjustment to situations
13. Tolerance of mistakes of others
14. Integrity (would he check a result without being told to?, etc.)
15. Check any of the following which might apply to this student:

Complainer
Ambitious
Overconfident
Lazy
Clockwatcher

Moody
Good sense of humor
Seclusive
Deceptive
Poor attendance

Poor health
Excitable
Suspicious
Unappreciative of the rights of others

List any outstanding or unusual qualities not covered:

List any special difficulties encountered by this student (handling the equipment, learning subject matter, following instructions, etc.):

Recommendations for improving performance:

Please give a brief, general evaluation of this student:

Would you hire this person to work with or under you?

With or without reservation?

If with, explain:

Date

Tardiness

Attendance

Instructor

APPENDIX L

FINAL EVALUATION RECORD

Date: ....

GRADE RECORD OF

....

at the completion of .... months course in

Grade

Orientation .... ——

Bacteriology .... ——

Serology .... ——

Parasitology .... ——

Hematology .... ——

Clinical Chemistry .... ——

Blood Banking .... ——

Routine Analysis .... ——

Basal Metabolism—Electrocardiography ——

Final Grade .... ——

Passing Grade .... ——

Grade Code:

A 90–100%
B 80– 90%
C 70– 80%
F Below 70%

Recommendations and Remarks: ....

....

....

Director of School

APPENDIX M

STUDENT FILE CARD ON GRADUATES

MEDICAL LABORATORY ASSISTANTS

Name ....

Address ....

....

Date entered:

Date completed:

Final average ....%

Out of ....

Class standing ....

Remarks and/or impressions:

Instructor ....

APPENDIX N

Laboratory Record Forms

The laboratory census or work tally is of prime importance to laboratory management and to hospital administration in estimating staff needs and predicting income potential respectively. It is also of strategic importance to persons concerned with planning medical laboratory assistant training. It can be used to evaluate learning experience potential, adequacy of teaching-service staff, and income potential related to requested investment in new equipment to improve service.

In order for the census to be effective for all these purposes, the rules for counting laboratory procedures should be consistent throughout the country. In 1954, the American Society of Clinical Pathologists proposed a set of rules for counting laboratory procedures. These rules were revised in 1957 and are in use in this revised form today. A complete set of ASCP *Forms for Reporting Clinical Pathology Tests, Surgical Specimens and Autopsies* (2nd Rev., 1957) can be obtained from Whiting Press, Rochester, Minn., for $4 per set of 25.

For simplification, the instructions and the form are oriented around six categories under clinical pathology: bacteriological, chemical, immunological, microscopical, physical, and others, plus the additional sections of surgical pathology and autopsies. The six categories are listed with the specific tests performed, and seven sources of material are listed across the top of the sheets: blood, urine, feces, CSF, gastric, sputum, and others.

The daily count of laboratory procedures is the responsibility of each laboratory section. The monthly report should be under the direction of the chief technologist. The monthly summary of the laboratory census, representing total tests per laboratory and the grand total for all laboratories, can be brief and still informative. An example of a total report follows:

GENERAL HOSPITAL
LABORATORY CENSUS
April 1964

Laboratory:	*Number of Tests*
Hematology-Urinalysis	2,500
Chemistry	2,020
Blood Bank	950
Bacteriology	1,822
ECG	600
Tissue-Pathology	1,270
Total	9,162

The yearly census, the total of all monthly census data, per test and per laboratory, should be completed at the close of the fiscal year. The format of this report can be the same as the monthly report form.

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